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THE DIAGNOSIS OF DECAY IN WOOD [1]

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INTRODUCTION

There are many industrial problems connected with the staining and rotting of wood. The questions asked by practical lumbermen could be answered much more satisfactorily if a reliable means were at hand for identifying the fungi producing decay in wood and wood products. The present study is an attempt to furnish a sound basis for future investigations leading toward more accurate diagnosis.

Although considerable work has been done by various investigators on many phases of the wood-rot problem, there is no literature dealing specifically with the diagnosis of decay. Early works on forest pathology were primarily histological studies, with a background of field observations on the life histories of the causal organisms. Both Theodor Hartig (*27*),[2] who as early as 1833 sought the cause of wood rots, and Willkomm (*90*), whose work appeared in 1866, believed in the spontaneous generation of fungi in wood. In 1878 Robert Hartig (*28, 29, 30*) showed that a certain fungus was responsible for a certain type of decay which had a fairly constant character in various species of wood. Later work in Germany was done by Falck (*22*) and Möller (*56*) on building rots, Czapek (*20*) on enzymic action, Münch (*57, 58, 59*), Mayr (*53*), Tubeuf (*79, p. 14–18, 31–44*), Neger (*60*) and others on biology of wood-rot fungi.

In England, Ward (*81, p. 287; 82; 83*), Biffen (*5, 6*), Bayliss (*4*) and Hiley (*35*) have contributed to the detailed information on wood rots and the fungi concerned. In Canada, Buller (*11, 12, 13, 14, 15*), Faull (*23, 24*), and White (*89*); and in the United States, Spaulding (*77, 78*), Schrenk (*68, 69, 70, 71, 72, 73*), Schrenk and Spaulding (*74*), Atkinson (*2*), Rankin (*61*), Long (*46, 47, 48, 49*), Meinecke (*54*), Weir (*84, 85, 86, 87*), and others (*1, 10, 33, 39, 41, 44, 65*) have given us valuable information on wood rots. The more recent contributions of Boyce (*7, 8, 9*) and of Kauffman and Kerber (*43*) deal mainly with incipient decay in wood. The papers in which cultural experiments with wood-rot fungi are presented have been comparatively few, that of Costantin and Matruchot (*17*) in France being one of the earliest. In some of the articles cited above, statements are found expressing belief that characters of decay may be used to identify the fungus causing the rot.

Since Hartig's time the presence of the fungus sporophore in close proximity to a specific decay in wood has been the single means of identifying that decay. However, the knowledge of wood rots has been gradually accumulating. The need for better diagnostic methods has become more and more apparent. Long and Harsch (*50*) have emphasized the importance of the cultural characters of fungi as diagnostic aids in establishing their identity. In the study here presented the author will attempt to show how gross, histological, and cultural characters may be applied to the diagnosis of decay in wood.

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[2] Reference is made by number (italic) to "Literature cited," pp. 565–567.

THE DIAGNOSIS OF DECAY

If a piece of wood which shows plainly that it is decayed has attached to it the fruiting body of a wood-rotting fungus, a probable diagnosis can be made by identifying the sporophore; but, if the sample bears no such fruiting body and shows little or no surface signs of decay, determining the causal organism is much more difficult. The writer's procedure is then as follows: First, the "unknown" is compared with a large number of "knowns." This has been made possible by the building up of a collection of some 600 or more typical rot specimens authenticated by the known identity of the sporophores collected on them. (Samples of wood rots collected from tree trunks which have lain on the ground for several years are not suitable on account of the possible presence of other wood-rot fungi in the same substratum.) These rots are grouped according to a classification presented below.

The next step is a study of the gross characters of the rot and comparisons with descriptive data on various rots. It is obvious that a collection and detailed descriptions of rots are essential in connection with studies of the gross characters. A key for the classification of wood rots will be the eventual outgrowth of these detailed descriptions.

Details of hyphae and cell structure in the infected wood are then studied under the microscope and the cultural characters of the organism, isolated from the infected wood and grown on artificial media, are observed. These cultures and the subcultures obtained from them are next compared with authentic stock cultures. At this point the identification of the organism can often be established with reasonable accuracy. A further test may be made to determine whether the fungus isolated from the unknown sample reproduces in sound sterilized wood an identical decay.

THE STAGES OF DECAY

Before the diagnosis can be discussed in detail, it is necessary to define the terms decay, decay stages, decay processes, and the classification of decays.

Decay (known to lumbermen as "dote," "doze," or "punky wood") is a process which begins with the development of wood-destroying fungi in wood and ends with more or less complete dissolution. The term includes all stages in the destruction process and two principal stages have been recognized by writers on wood decay. The early development of decay has been termed: Beginning, early, initial, incipient, advance, immature, first, primary, and invasion stage; and the final or decomposed stage: Final, last typical, advanced, mature, complete, ultimate, destruction. The two descriptive terms, invasion stage and "destruction stage," were extensively used by Falck (*22*). The terms "incipient" and "typical" have been selected by the writer to represent the two stages.

THE INCIPIENT STAGE

The incipient stage of decay corresponds to the period when the mycelium of the fungus is invading new host tissues preparatory to a more complete attack upon the cells (fig. 1, I). During this stage the hyphae ramify in all directions. In most decays the infected wood exhibits color changes although to the naked eye it appears to retain its structural characters, not having become punky, soft and spongy, stringy, ring-shaked or pitted. In some rots a softening of the wood may be detected in this stage and a characteristic brashness is evident, though with little or no discoloration.

Incipient discolorations and zone lines (*62*, *63*) usually accompany the incipient stage of decay. These are generally due to the presence of by-products resulting from the dissolution of the cell walls and cell contents by the fungous enzymes.

THE TYPICAL STAGE

The typical stage is that in which decay is plainly a factor in breaking down the wood cells. With few exceptions it is accompanied by unmistakable changes in strength properties of the infected wood, changes in color and changes in continuity and texture (fig. 1, T). One of the exceptions noted above is *Trametes pini* when the white pits are few and wide apart and the tissues are heavily infiltrated (*16*). Since infected wood undergoes color changes, the nature of the attacking fungus determines whether the rotted wood becomes whitish because of delignifying action, bleaching, or other causes, or whether the infected tissues become yellow, red, or brown due to a cellulose-dissolving action or to other causes not yet fully understood. The continuity of the wood tissues is broken in most decays in this stage. The wood

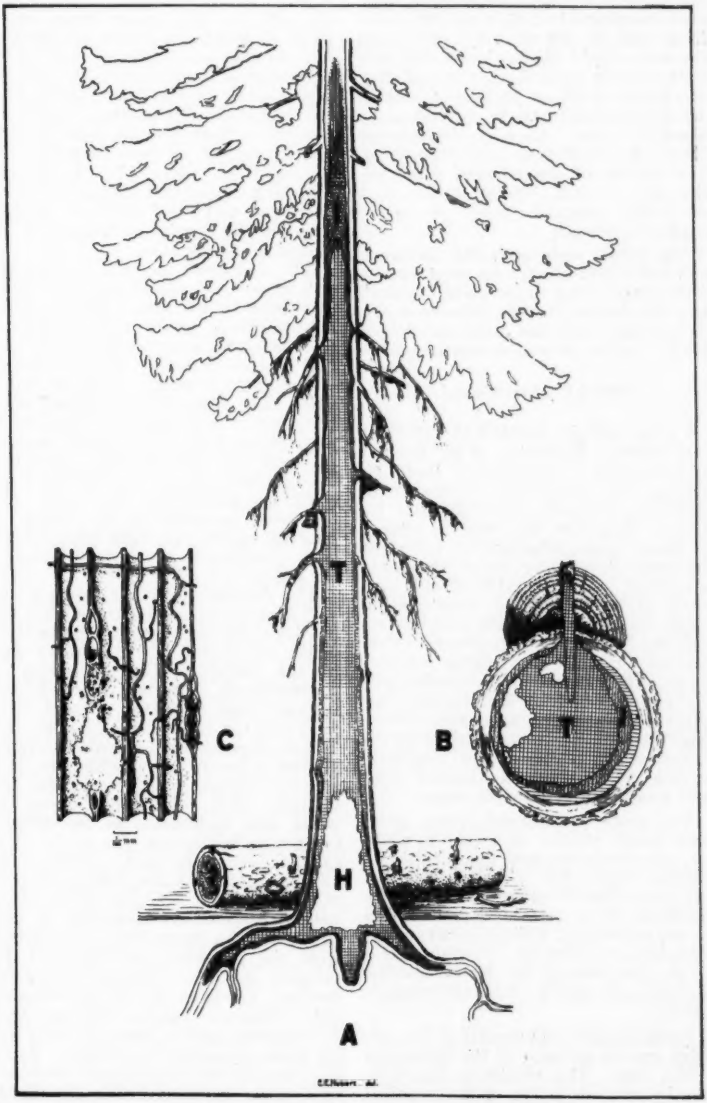


FIG. 1.—Heart rot in *Tsuga heterophylla* caused by *Echinodontium tinctorium* (Indian paint fungus). A.—Section through living tree showing the extent of decay with hollow cavity at H, typical rot at T, incipient rot at I, hidden phase of incipient decay beyond. The rot extends into the larger branches. B.—Cross section of the trunk showing relation of decay to sporophore and dead branch. C.—Tangential section of decayed wood showing the hyphae of the fungus penetrating the cell walls and decomposing them. Note the numerous bore holes.

may become soft and spongy and the remaining tissues become collapsed; and the result may be a stringy, spongy mass merging into hollow rot (fig. 1, A). Ring scale or ring rot may result from the more rapid dissolution of the cells in the spring wood of each annual ring, or a group of pits or pockets of isolated decayed tissues may be formed as in *Trametes pini*, *Stereum frustulosum* (Pers.) Fr., or *Polyporus amarus* Hedg. The texture of the infected wood varies from a soft, spongy mass to a shrunken, cracked mass of easily crushed material.

The terms *early incipient*, *incipient*, and *late incipient* may be used for the early stages, and *early typical*, *typical*, and *late typical* for the later ones, although hard and fast lines can not be drawn, as the process is continuous.

DECAY PROCESSES

Under certain conditions wood is very stable. Exclusion of air from it by immersion in water, or by thorough drying and subsequent exclusion of moisture, prevents decomposition by decay. However, all wood is subject to decay when attacked by the right organism developing under favorable conditions. Both heartwood and sapwood are attacked by wood-rotting fungi, the sapwood usually proving less resistant. Similarly, on account of its thinner cell walls and greater porosity or its different chemical composition, the spring wood of the annual ring offers less resistance to fungous attack than the summer wood. This often results in a type of decay called ring scale or ring rot, in which the annual rings split apart in a cylindrical plane.

The more pronounced decay processes result usually in either a white rot, or a brown rot, and this furnishes a general basis for classification. In some rots the two processes seem to be combined to a certain degree and a brown rot results, which contains areas resembling a white rot. Certain stages of the rot caused by *Echinodontium tinctorium* E. and E. show this characteristic.

Delignification apparently is the primary process in many of the white and brown rots. This results in the splitting by enzymic action of the so-called lignin-cellulose compound into a lignin complex (composed of hadromal, coniferin, and vanillin) and a cellulose complex (a polymer of starch). It rests with the particular attacking fungus whether the lignin complex is all or in part absorbed and the cellulose complex left (white rots) or whether the reverse action takes place (brown rots) (*11*, *20*, *28*, *63*). Some fungi are capable of absorbing both compounds in varying amounts, as will be shown later.

In some of the white rots the enzymic action continues and in the typical stage the lignin complex is often entirely removed, so that the presence of aldehydes can not be demonstrated with the use of indicators (*18*). In others a bleaching action takes place as the rot develops and the typical reaction wherein the lignin compound is removed seems lacking.

The cellulose complex is removed and apparently absorbed by the fungi producing brown rots. This cytolytic action usually leaves a brown, friable mass resembling charcoal in brittleness and composed mainly of the elements of the lignin complex. The decomposition may proceed by an attack upon the tertiary wall first and advance to the primary wall, or the process may be reversed. In these processes all stages from partial to complete disintegration of the cell walls are to be found. The bore holes produced by the hyphae in penetrating the cell walls are evidence of a complete localized destruction of the wall.

The dissolution of the primary cell wall may take place before or after the cytolytic reaction begins. Separation of the two halves of the primary wall in the incipient stage of decay is characteristic of certain rots. In the typical stage of other rots it may represent the last phase. An enzyme, pectase or pectinase, has been described as the ferment responsible for this action in various plant tissues (*20*, *42*). Czapek (*20*), discussing decay in wood, lists hadromase as the delignifying enzyme; cytase, the enzyme attacking cellulose; and pectase, the enzyme attacking the middle lamella of the cell wall. Schmitz (*66*) working with *Echinodontium tinctorium* E. and E. isolated a dozen different enzymes from the powdered mycelial mat developed in pure culture on carrot media. The same writer (*67*) working with *Polyporus volvatus* Pk. recorded 12 enzymes; and he listed 13 enzymes for *Fomes igniarius* (L.) Gillet. Zeller (*91*) determined the enzymes produced by *Lenzites sepiaria* growing on artificial media. A specific type of decay is thus produced in wood by a specific fungus; and a certain species of fungus produces quite similar decays in different species of woods, with occasional minor variations.

THE CLASSIFICATION OF WOOD ROTS

Very often decays are placed in two main divisions, heart rots and sap rots, on the basis of the parts of the wood attacked. The sap rots may be again divided into the decays of the live sapwood, such as those produced by *Polyporus dryadeus* or *Fomes igniarius*, and the decays of the dead sapwood and also heartwood, such as that produced by *Polystictus abietinus* Dicks. in conifers.

Frequently the heart rots are classified with respect to the longitudinal portion of the tree attacked. The decays found in the main portion of the tree are termed trunk rots (fig. 1, A); those found in the upper trunk, top rots; those found in the lower, basal portion, butt rots. The butt rots usually extend some distance into the heartwood of the roots (fig. 1, A). The decay produced by *Polyporus schweinitzii* Fr. is of this type. The term root rot is usually applied to a sap rot of live roots of which the *Armillaria mellea* (Vahl.) Quel. decay is a good example.

A classification based upon the particular reaction produced in the wood by the fungus seems of greater value in decay diagnosis. There are two groups due principally to the particular chemical or enzymic reaction of the fungus with the wood, which show the color distinction mentioned above (p. 526).

The white rots are caused mainly by the lignin-dissolving fungi, although they are not restricted to this type. They are usually whitish in the areas where the decay has reached the typical stage. These areas are principally composed of white cellulose compounds or are bleached to a whitish color as a result of the fungous action. The white rots may be divided into white pocket rots, white ring rots, white mottled rots, and white spongy rots (Pl. 1, A, B, C). The second group, comprising the brown rots, caused mainly by cellulose-dissolving fungi (but not limited to these), shows yellowish, reddish, or brownish discolorations in the typical stage. Usually the cellulose has been extracted for food by the fungus, leaving the lignin compounds in the remaining wood tissues. Under the brown rots of the coniferous woods the subgroups are brown pocket rots, brown ring rots and stringy rots, brown cubical rots, brown spongy rots, and brown mottled rots (Pl. 2, A, B, C).

Of the fungi treated in this study, *Trametes pini* (Brot.) Fr. (*Fomes pini*) (Brot.) Lloyd and *Polyporus anceps* Pk., represent the white pocket rots. *Fomes igniarius* Linn. represents the white spongy rots; *Echinodontium tinctorium* E. and E. represents the brown ring rots and stringy rots; *Pholiota adiposa* Fr. represent the brown mottled rots; *Polyporus amarus* Hedg. represents the brown pocket rots; and *Polyporus schweinitzii* Fr., *Polyporus balsameus* Pk., *Lenzites sepiaria* Fr. *Trametes carnea* Nees. and *Lentinus lepideus* Fr. represent the brown cubical rots. The principal groups of wood rots are thus represented. More brown cubical rots than other types are discussed because this group appears to contain rots the incipient stages of which are often not distinguishable by discolorations of any kind, and which for this reason present a serious problem from the economic viewpoint.

Certain fungi produce in wood cells changes slightly resembling those found in true decay (*88*). These are the wood-staining or sap-stain fungi, of which the blue stain fungi, *Ceratostomella* spp., are representative (*34*). Their hyphae have been observed penetrating the tracheid walls in the woods of pine and other species (*36*, *37*, *57*, *72*). Penetration is not frequent, however, and is not so prominent a decomposition process as that produced in the medullary rays where the walls of the ray cells are often completely broken down. More commonly the hyphae in passing from cell to cell seek the natural openings, the bordered pits, where the netlike perforated membrane supporting the torus apparently presents an easy passageway, or they pass through the simple pits in the cell walls.

GROSS CHARACTERS OF DECAY

Color.—The discolorations usually associated with the stages of a particular decay are the most valuable diagnostic characters visible to the naked eye (Pls. 1 and 2). They vary greatly for different fungi and slight variations may be noted for the same fungus in different hosts. Color forms the basic part of rot descriptions, but these descriptions, of course, can not be presented in detail here. However, the discolorations produced in wood by the advancing hyphae of wood-rotting fungi should be given more than passing attention because it is the incipient stage that in many cases eludes detection.

Only a few important rots have striking or even visible incipient discolorations. These are more common in

white rots, but quite absent in brown rots, particularly brown cubical rots and brown pocket rots.

The incipient discoloration produced in wood by *Trametes pini* (Pl. 3, A) exemplifies the invasion discoloration found in many white pocket rots. It is so common in the lumber and timber of commerce that it is known as "red heart" or "firm red heart." It occurs in nearly all species of coniferous wood and is almost entirely confined to the heartwood. According to the host and the conditions of development of the fungus, the "red heart" varies from pinkish to dark reddish or brownish or sometimes a purplish color. While the appearance of a distinct color in the wood may indicate the presence of decay, identification is complicated by the fact that such fungi as *Polyporus anceps* Pk. and *Fomes annosus* Fr., which produce white pocket rots, also cause pinkish to reddish discolorations in the incipient stage of rot. *Polyporus circinatus* has recently been collected in Minnesota on roots of living *Picea mariana*, producing in this wood a white pocket rot having a light to dark reddish incipient discoloration. This observation, verified by cultures, adds another to the list of fungi producing reddish discoloration in heartwood.

The incipient stages of certain brown rots, such as produced by *Fomes laricis*, *Polyporus sulphureus*, *Polyporus schweinitzii*, *Fomes pinicola*, and others, in certain hosts may often be confused with each other or with the invasion discolorations produced by the white pocket-rot fungi. The natural pinkish or reddish color of the heartwood of certain hosts, such as *Pseudotsuga taxifolia*, adds to the confusion. Certain invasion discolorations take on the nature of a water-soak in the infected wood of freshly cut trees. This is characteristic of the incipient stages of the white rots produced by such fungi as *Ganoderma tsugae* in *Tsuga canadensis* (Pl. 3, B), and *Polyporus dryophilus* in broadleaf hosts. According to Long (*46*, *48*), *Polyporus pilotae* and *Stereum subpileatum* also produce a water-soak in the incipient stage of decay. In the case of *Ganoderma tsugae* the water-soaked areas soon lose their definition on drying, and little or no discoloration remains to indicate the incipient decay areas.

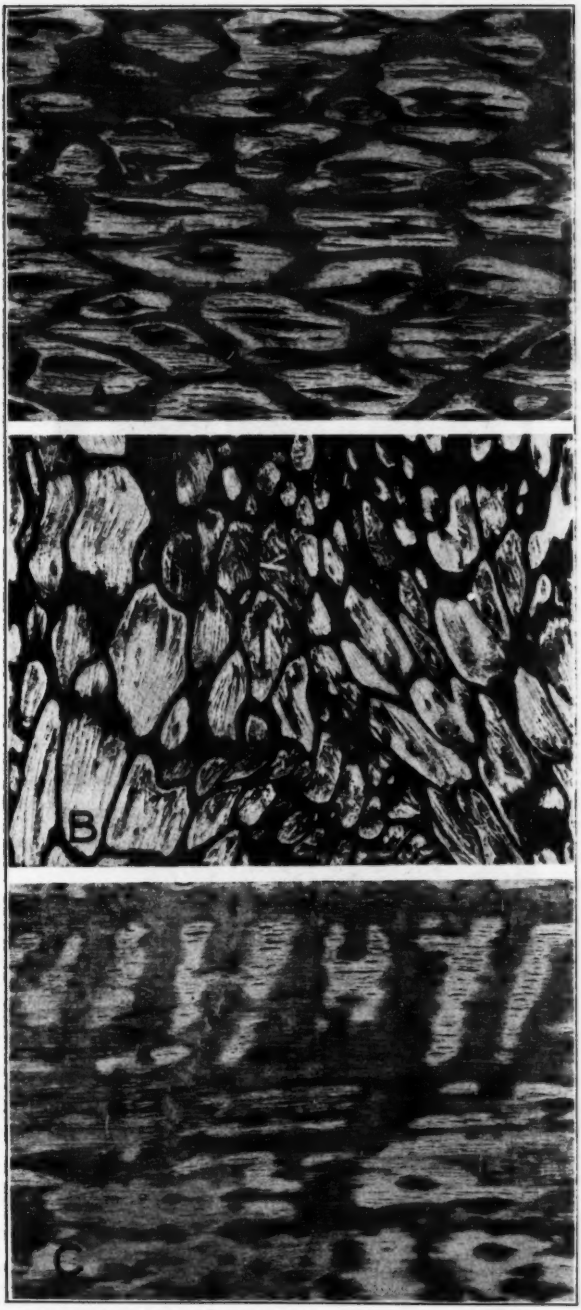
In the incipient stage of decay produced by *Echinodontium tinctorium* the incipient discolorations extend some distance ahead of the typical stage (fig. 1 and fig. 2, B). Where the discolorations fade out and for some distance beyond this limit, the action of the fungus on the wood structure continues. Consequently, sound-appearing boards cut from infected areas in the logs show a brashy, crumbly texture which unfits the stock for commercial use when seasoned (*9*, *54*, *85*). *Polyporus anceps*, probably identical with *Polyporus ellisianus* as used by Long (*49*), produces in *Pinus ponderosa* a characteristic incipient discoloration (fig. 2, A), which so far has not been observed in other hosts of the fungus. Reddish to reddish-brown areas radiate from typical rot areas viewed in transverse section. Lighter reddish discolorations have been noted in *Picea canadensis* infected with *Polyporus anceps*. White (*89*) states that when *Fomes applanatus* develops in living hosts the incipient decay is bordered by a brownish invasion zone one-fourth inch wide or wider.

White considers this character is of diagnostic value. *Fomes igniarius* produces in the incipient area a characteristic invasion zone, usually a dark band or zone of irregular outline and width (fig. 2, C). *Pholiota adiposa* produces in *Tilia americana* a dark brown, characteristic invasion zone which is often included within the area of typical decay as the invasion progresses (fig. 2, D). Pronounced incipient discolorations are not observed frequently in the brown rots. The rot caused by *Fomes pinicola* in *Tsuga heterophylla* is an example, showing but a faint discolored area of incipient decay which does not form a continuous band around the typical rot areas (fig. 2, E). Some incipient and typical discolorations common to commercial woods are well illustrated in the work of Boyce (*9*).

The difference between white and brown rots in the typical stage is usually very clear. White rots commonly show distinctive incipient discolorations and brown rots do not. Hence brown rots probably cause greater economic loss. The difficulty in diagnosis often lies in distinguishing decays in which the incipient discol-

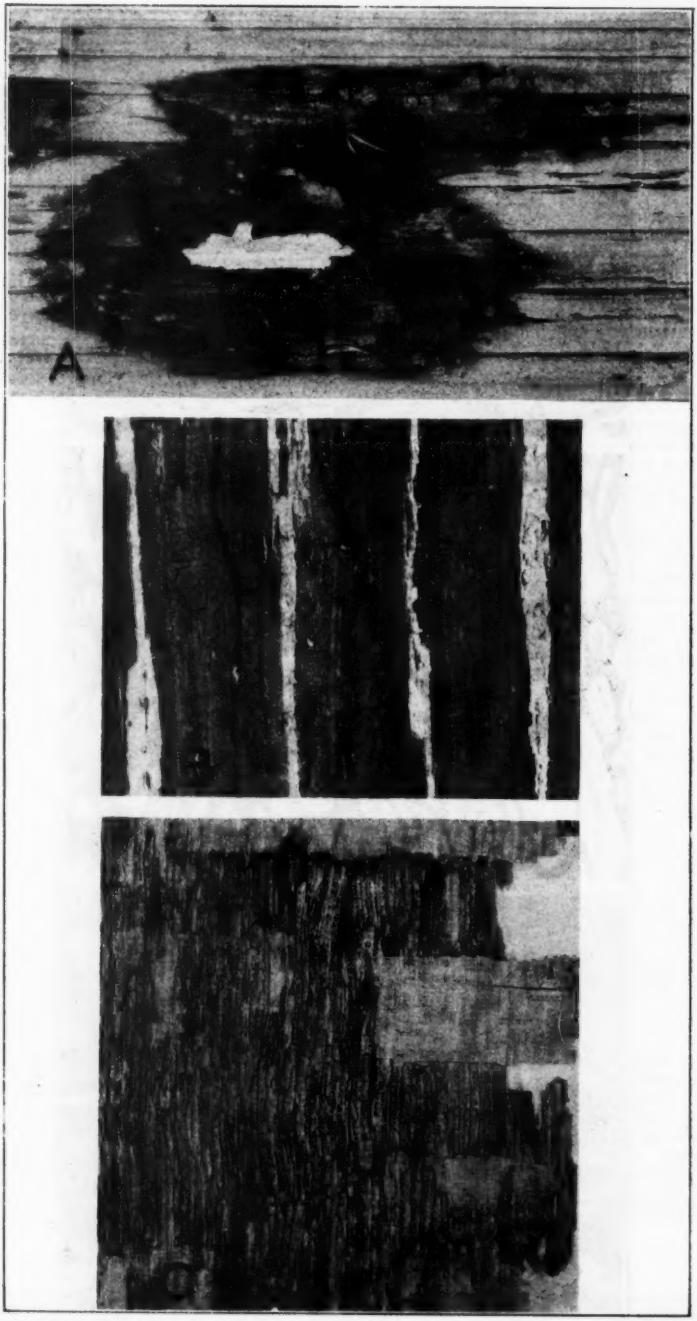
EXPLANATORY LEGEND FOR PLATE 1

Three typical white rots (about natural size). A.—White pocket rot caused by *Fomes nigrolimitatus* (*F. putearius* Weir) in *Larix occidentalis*. Tangential section. B.—White pocket rot caused by *Fomes extensus* in a hardwood root (tangential section). C.—White mottled rot caused by *Fomes applanatus* in *Populus grandidentata*. Radial section. Dark-colored zone lines often accompany this rot.



The Diagnosis of Decay in Wood PLATE I

(For explanatory legend see p. 528)



The Diagnosis of Decay in Wood

PLATE 2

(For explanatory legend see p. 531)

orations as well as the typical characters resemble each other closely.

ZONE LINES.—Thin zones of discolored matter usually appearing in cross section as narrow to broad lines, particularly those associated with incipient decay, are valuable diagnostic characters (Pl. 4), though additional evidence is usually necessary. They are usually formed in the white rots only, being rare in brown rots. Their cause is still unsettled. Rhoads (*62*) concludes that the brown substance (decomposition products) commonly occurring in dicotyledonous woods attacked by wood-destroying fungi arises only after the death of the cells through the oxidation of their contents and certain constituents of their walls, occurring most notably in the parenchyma cells.

Observations on zone lines formed between two fungi occupying the same wood tissues indicate that this type of zone line is fairly common. But in several cases these zone lines differed from the clear-cut, brightly colored, narrow lines formed through desiccation, which will be discussed later. The zone lines between the rots produced by *Polyporus anceps* and *Lenzites sepiaria* in *Picea canadensis*, for example, are broader, up to one-fortieth inch wide, with indistinct edges and of a dull brownish color. Very often a double zone line is formed. The zone lines between rots of *Fomes applanatus* and *Stereum frustulosum* in *Quercus* sp. resemble the above closely, but are fainter and less distinct. The zone lines of *Fomes applanatus* are usually narrow, brownish black to black, sharply defined, and rarely doubled.

Schrenk (*73*) mentions the orange-colored zone lines accompanying the decay produced by *Polyporus adustus* in the sapwood of red gum (*Liquidambar styraciflua*). The zone lines produced in wood inoculated with pure cultures of a fungus (Table I) isolated from sap-gum boards showing a white rot with orange zone lines, and produced in pure cultures on malt agar are identical in width and color with those found in infected sap-gum lumber. However, the typical rot produced in this experiment is a white spongy rot and in the writer's experience the typical rot of *Polyporus adustus* in certain broadleaf hosts is always a white mottled rot and is very characteristic. Whenever the white mottled rot was observed in connection with sporophores of *Polyporus adustus*, the orange zone lines were lacking. This would indicate that some fungus other than *Polyporus adustus* may be responsible for the white spongy rot and orange zone lines in sap gum.

Some writers (*84*, *89*) attribute the zone lines in infected wood to reactions between two wood-inhabiting fungi occupying the same substratum. White (*89*) in his description of the rot produced by *Fomes applanatus* states that—

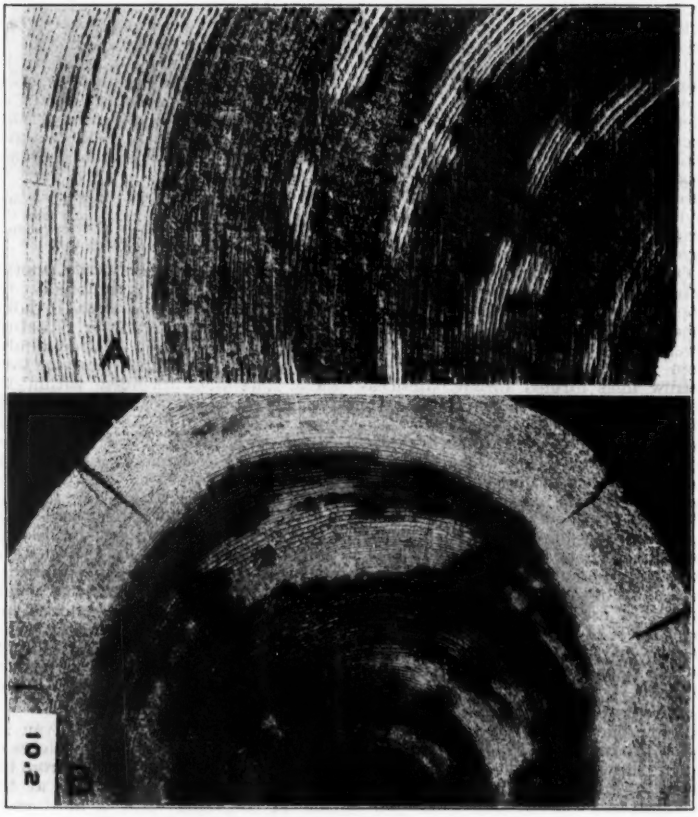
> black lines are encountered but cultural or other evidence shows that when they do occur more than one species of fungus is at work. They are produced at the point of contact of two invading fungi. * * *

If this proves generally true, another type of zone line undoubtedly not caused by contact of two invading fungi must be accounted for. A study of the characteristics of a large number of wood rots shows that many of them have zone lines included within the typical rot areas (Pl. 4). Although such areas, including zone lines, were cultured repeatedly, no fungus other than the one responsible for the decay developed in any case. Field observations indicate that these zone lines occur more commonly in the pieces of infected wood most subject to desiccation. Hartig (*28*, *p. 32–39*) states that the zone lines in oak wood infected with *Fomes igniarius* (*Pl. 15, fig. 2*) are due primarily to the entrance of air into the infected wood tissues. Lindroth (*45*) figures a zone line formation just back of the freshly-cut surfaces of birch wood infected with *Polyporus nigricans*.

Additional observations, given in Table I, were made on large pieces of infected wood brought in fresh from the woods. The pieces when cut at the time of collecting were in most cases completely infected but showed no zone lines; when cut open after a period of drying in the warm air of the laboratory, many of the rots showed typical zone lines extending transversely across and but a short distance back from the ends of the infected log sections. Desiccation and oxidation of the decomposition by-products seem responsible for the formation of these lines. Data

EXPLANATORY LEGEND FOR PLATE 2

Three typical brown rots (about natural size). A.—Brown pocket rot caused by *Polyporus amarus* in *Libocedrus decurrens*. "Pecky" incense cedar (radial section). B.—Brown cubical rot caused by *Fomes pinicola* in *Tsuga canadensis*. Note the white mycelial mats in the cracks. Radial section. C.—Brown spongy rot caused by *Polyporus berkeleyi* in *Quercus alba*. "String and ray rot." The rays are left intact and form a remarkable contrast to the crumbly, spongy condition of the adjoining tissue. Radial section.



The Diagnosis of Decay in Wood **PLATE 3**

A.—Transverse section of southern yellow pine wood infected with *Trametes pini* showing the characteristic invasion or incipient rot area preceding the typical rot containing pockets (about natural size).

B.—Transverse section of a log of *Tsuga canadensis* infected with *Ganoderma tsugae* showing the dark "water soak" appearance of the incipient stage of the rot. Photographed without color screen, soon after cutting (about $\frac{1}{5}$ natural size).

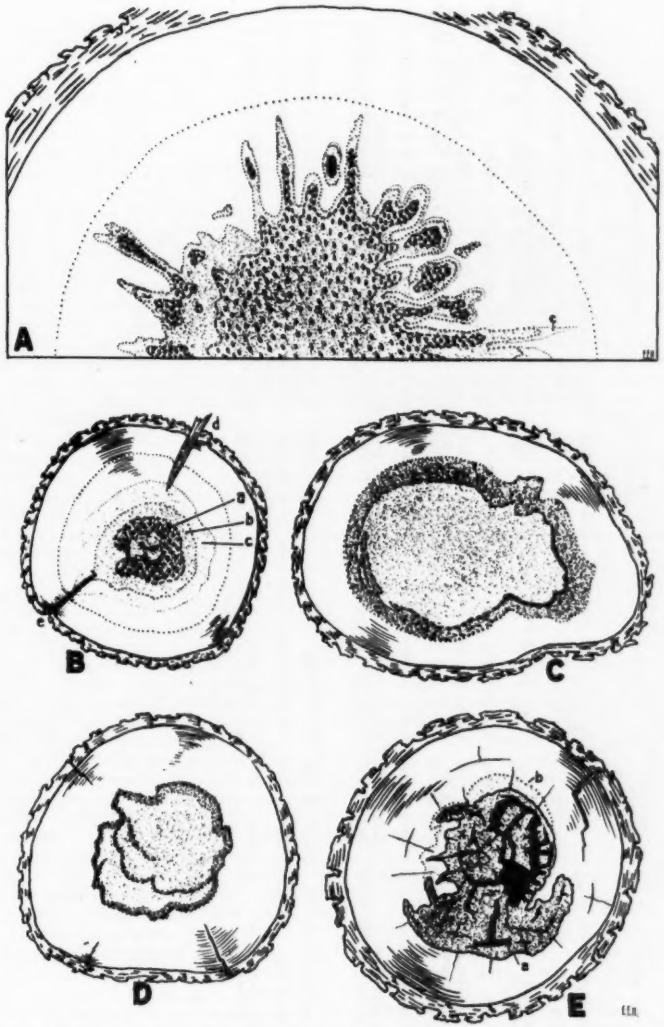


FIG. 2.—A.—Transverse section of *Pinus ponderosa* infected with *P. anceps* (*P. ellisianus*). Typical grayish-white pocket rot at *a* and similarly shaded isolated areas. Red-brown incipient rot at *b*. At *c* a faint yellow-brown invasion zone which appears only in part of the infected wood. Note the radiate development of the rot. One-fifth natural size. B.—Transverse section of *Tsuga heterophylla* infected with *Echinodontium tinctorium*. Typical brown stringy rot at *a*, typical rot not stringy in texture at *b*, typical rot grading into incipient rot at *c*. A very faint discoloration extending from incipient area at edge of *c* up to sapwood zone at dotted line. Dead branch stub at *d* and unhealed frost crack at *e*. One-sixteenth natural size. C.—Transverse section of *Acer rubrum* infected with *Fomes igniarius*. Typical rot in central shaded area surrounded by the darker band of the invasion zone. One-sixteenth natural size. D.—Transverse section of *Tilia americana* infected with *Pholiota adiposa*. Typical rot in central shaded area surrounded by the darker band of the invasion zone. Note the earlier invasion zones still showing and included within the typical rot area. One-sixteenth natural size. E.—Transverse section of *Tsuga canadensis* infected with *Fomes pinicola*. Typical brown cubical rot at *a*. Faint yellowish-brown discolored areas of incipient rot at *b*. One-sixteenth natural size.

TABLE I.—*Formation of zone lines in various species of infected wood after drying*

Fungus	Host	Date collected	Condition	Date examined	Zone lines across ends of sample
Fomes applanatus	*Acer saccharum*	July 1921	Fresh; mine timber; no zone lines.	Nov. 3, 1921	Narrow, continuous, dark brown, ⅛ to ¼ inch back from surfaces.
Do	*Malus* sp	1918	Old specimen; no zone lines.	Aug. 16, 1921	Narrow, brownish black parallel to cut surfaces, $\frac{1}{16}$ to ⅛ inch back from surfaces.
Do	*Hicoria ovata*		Fresh; no zone lines	Mar. 8, 1922	Narrow, black, $\frac{1}{16}$ inch back from one end of sample.
Do	*Acer negundo*	Nov. 26, 1922	do	Dec. 7, 1922	Narrow, continuous, black to brownish, ⅛ to ¼ inch back from surfaces.
Fomes everhartii	*Quercus* sp	May 18, 1921	No zone lines	June 10, 1921	Narrow, black, irregular, not parallel, ½ to 1 inch back from surface.
Fomes fomentarius	*Betula occidentalis*	Sept. 1921	Fresh; no zone lines	Dec. 2, 1921	Narrow, brownish black, $\frac{1}{32}$ inch back from surfaces.
Fomes igniarius	*Ulmus americana* logs	Sept. 12, 1921	do	Nov. 17, 1922	Narrow, continuous black, ½ inch back from ends of logs.
Echinodontium tinctorium	*Abies grandis*	Apr. 24, 1923	Fresh; zone lines in typical rot areas.	May 12, 1923	Reddish, irregular lines along edges of loose fibrous areas of rot.
Ganoderma tsugae	*Tsuga canadensis*	Sept. 17, 1921	Fresh; no zone lines	Oct. 12, 1921	Narrow, continuous black, ¼ to ½ inch back from one end surface.
Polyporus betulinus	*Betula papyrifera*	Sept. 17, 1921	do	Nov. 11, 1921	Zone lines observed across ends of sample.
Schizophyllum commune and *Polystictus pargamenus.*	*Populus grandidentata* branch	June 21, 1921	Fresh; zone lines parallel to branch.	June 27, 1921	Narrow, continuous, brownish black, almost parallel to surfaces, ¼ to ½ inch back from surfaces of both ends.
Stereum sulcatum	*Larix occidentalis*	Apr. 24, 1923	No zone lines	May 15, 1923	Narrow, black, roughly parallel to ends, ¼ inch back from surfaces.
Trametes pini	*Picea canadensis*	June 1921	Few lines in typical rot areas.	Nov. 23, 1921	Narrow, dark brown to black, ⅛ to ¼ inch back from end surfaces.
Do	*Pinus strobus*	Sept. 1921	No zone lines	July 8, 1922	Narrow, dark brown, ⅛ inch back from end surfaces.
Do	*Picea sitchensis*		Inoculated with pure culture; the inoculated block dried out.	Nov. 23, 1921	Narrow, continuous, across both ends of blocks.

on the appearance of zone lines in wood inoculated with pure cultures of wood-destroying fungi support this view. The zone lines invariably appeared during the periods when the moisture in the tubes and in the inoculated blocks had nearly evaporated. Zone lines were secured in the following cultures on the hosts named: *Trametes pini* in *Picea sitchensis* heartwood, *Fomes igniarius* in *Populus tremuloides* sapwood, *Xylaria polymorpha* in *Tilia americana* heartwood, *Polyporus adustus* (?) in *Populus tremuloides* sapwood, *Hymenochaete rubiginosa* in *Populus tremuloides* heartwood, *Fomes applanatus* in *Populus tremuloides* sapwood, *Ganoderma curtisii* in *Populus tremuloides* sapwood and in pure cultures on malt agar, the mycelium extending to the cotton plug and forming a zone line across the plug.

The data in Table I indicate that whenever freshly cut pieces of wood infected with certain fungi are placed in a dry room or left to dry in the open, characteristic zone lines are formed a short distance back and parallel to the cut surfaces (Pl. 4, A, B, C). Field observations often show zone lines in the upper portion of rotted stumps in the region where excessive drying occurred, roughly parallel to the surfaces from which the evaporation took place.

Zone lines may be regarded as additional evidence or as aids in diagnosing decay, but in most cases can not be regarded as infallible characters in determining the causal organism. The data indicate that one type of zone line is commonly formed during desiccation. Other types, less common, have various origins. The entire problem of zone lines needs to be attacked from the microchemical angle in order to determine the fundamental causes for these characteristic despoits. In this way their diagnostic value may be more fully developed.

TEXTURE.—Texture may be determined by hardness, elasticity, brashness, or ease of crushing or crumbling. Hardness may be tested in a number of ways, but the comparative resistance offered when a knife blade or a dull point is jabbed into sound and decayed wood has been found fairly satisfactory. Prying up slivers of the wood with a knife blade often furnishes comparative evidence. This common test for elasticity in general use by inspectors (7) is of value since the sounder wood pulls out with a more splintery appearance and shows greater elasticity and coherence than the infected wood. The manner in which fibers tear out at the saw cuts is also a reliable sign of decay.

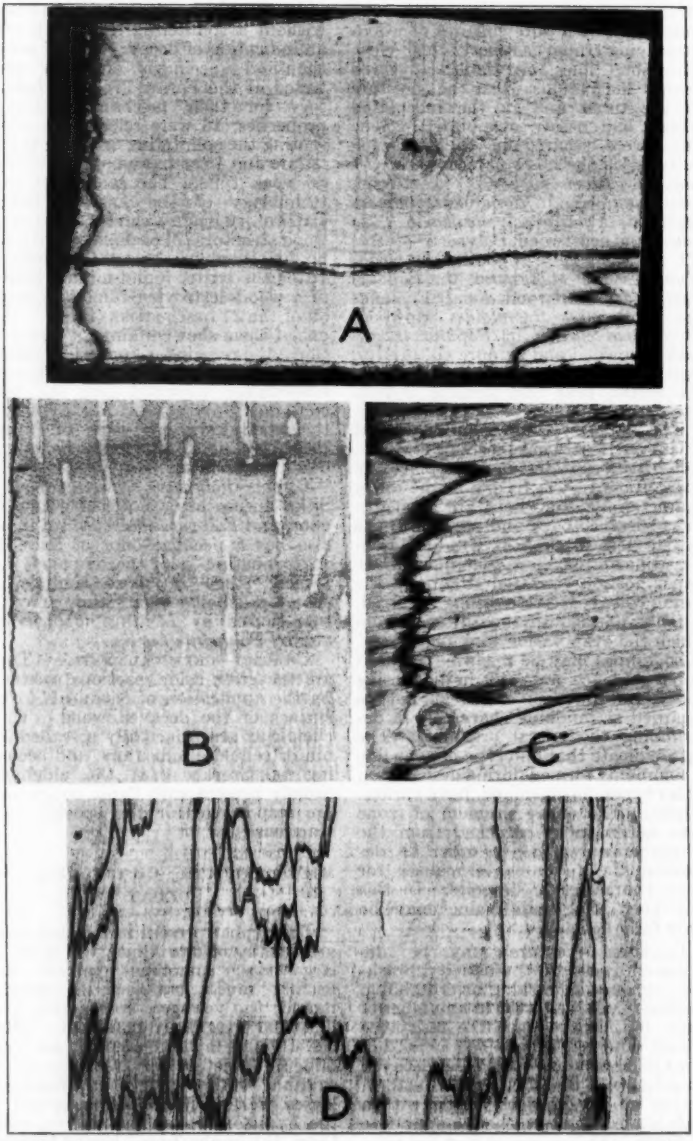
BRASHNESS.—The tendency to break without a splintering fracture is often an indication of decay. Out of several thousand specimens of Sitka spruce tested at the Forest Products Laboratory for their mechanical strength properties, 16 were selected as representing the splintering or tough type of failure and 15 as representing the brash or weak type. The section of wood technology of the Forest Products Laboratory made a careful study of the wood structures of each piece and found no definite physical cause for brashness. Later the writer found minute hyphae of a wood-destroying fungus in nearly all of the 31 brash sticks (Pl. 5). Only one of them showed faint rot discolorations, the remainder appearing normal. Two of the tough sticks contained blue-stained sapwood and the third contained only a very few hyphae of the wood-destroying type of fungus. Brashness may, of course, be due to other factors also.

ODOR.—*Polyporus schweinitzii* rot in Sitka Spruce has a noticeable anise-oil odor, and *Polyporus berkeleyi* rot in oak also has a peculiar odor of anise oil. A turpentine odor accompanies the typical stage in most conifers. A somewhat similar odor accompanies the rots caused by *Lentinus lepideus* Fr. and by *Polyporus fissilis*.

CHROMO-CHEMICAL TESTS. — These are the gross color reactions produced by the application of chemicals to the surface of the decayed wood. These chemicals are principally so-called lignin or cellulose indicators, and according to Crocker (*18*) the aldehydes usually present with lignin compounds are responsible for the specific color reactions.

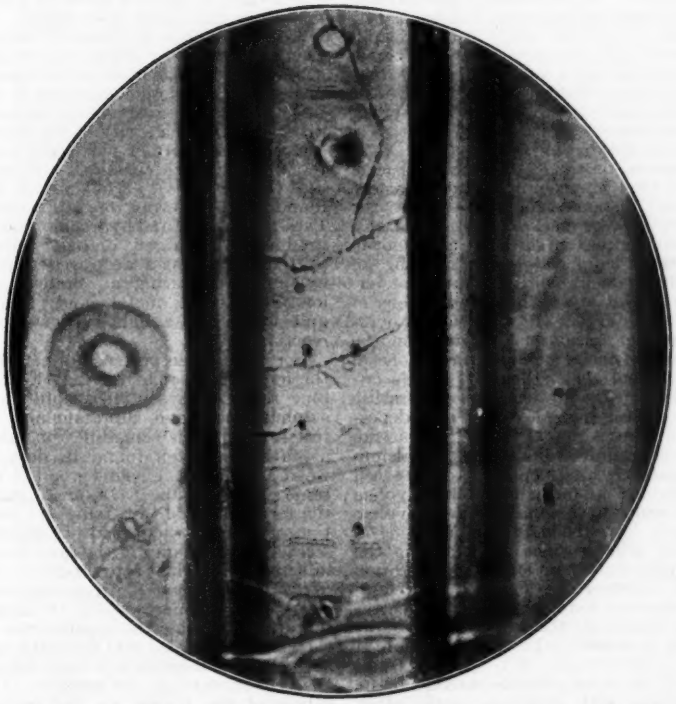
MICROSCOPICAL CHARACTERS OF DECAY

The hyphae present in infected wood are usually of two kinds, the older, of large size, sometimes colored and usually much branched or anastomosed; the younger, very small, hyaline, and irregularly branched. Often these young hyaline hyphae are very difficult to detect even with the aid of suitable stains and oil immersion lenses (Pl. 5). With the exception of the hyphae of some of the sap-stain fungi, the hyphæ found boring through the cell walls are rot producers. Hyphae may be found in the medullary rays, in tracheids, in vessels, in sapwood or in heartwood, and may be colored or hyaline. Clamp connections sometimes called buckles or nodose septae usually indicate a wood de-



The Diagnosis of Decay in Wood **PLATE 4**

A.—Zone lines in living *Betula papyrifera* infected with *Fomes fomentarius*. B.—Zone line developed in drying *Acer saccharum* infected with *Fomes applanatus*. Note the direction of the line paralleling the freshly cut end. C.—Zone line in *Picea canadensis* infected with *Trametes pini*. The line follows irregularly behind the cut and extends inward where a knot intervenes. D.—Zone lines produced in fallen trunk of *Acer rubrum* by *Xylaria polymorpha*. Zone lines are formed around the decayed areas which originate from various foci of infection, producing double lines. About ½ natural size.



Photomicrograph showing minute hyaline hyphae of a wood-destroying fungus (*P. schweinitzii*) in a test piece of *Picea sitchensis*. Note the penetration of the cell wall as indicated by the darkened circular areas in the path of the hyphae ×1300. Stained with Bismark brown and methyl violet.

stroyer. So-called medallion hyphae are common to *Lenzites* spp. (*22*). Granular deposits on the outer walls of the hyphae are characteristic of certain species of Peniophora or Coniophora and sometimes of *Lentinus lepideus* (*11*, *22*).

The order of dissolution of the cell wall may often suggest the type of decay. If the primary wall is resistant the bore holes may be constricted (19 in fig. 6). Spiral cracks in primary and secondary walls and bore holes or cell-wall punctures are often reliable characters; also spiral shrinkage cracks extending from pit openings or bore holes (fig. 5, E, F). The latter have also been found in wood in which hyphae were absent. If it is proved that these cracks are due to fungous action, their presence in tissues lacking hyphae of any kind may point to enzymic action some distance removed from the secreting hyphae. Corrosion marks, grooves, uneven surfaces, and uneven thicknesses of cell walls, splitting of middle lamella, and dissolution of tertiary layer are all good indications of decay. White (*89*) considers overabundant formation of tyloses in the region of incipient decay, and none in the sound region beyond, a safe criterion of the presence of a parasitic wood-rot fungus. Wound gum deposits in the region ahead of hyphae are often characteristic signs. Calcium oxalate crystals deposited on the cell walls and in form resembling hyphae may indicate (*Lentinus lepideus*) hyphae which have been decomposed (*11*). Other reliable signs of decay are the enlargement of pit openings, corrosion and cracking of bordered pits, and presence of granular deposits or corrosion marks on the scalariform bars at ends of vessels. The accumulation in the bordered pits of by-products and resins in certain woods should not be confused with corrosion marks often seen on the embossed portion of these pits.

Corrosion, thinning, and entire decomposition of the cross walls are prominent decay characters in the medullary rays. Whether pit openings or bore holes are used as channels for hyphae may aid in distinguishing wood destroyers from staining or molding fungi. In the ray cells, decomposition products, wound gum, granular deposits, and tannin are often of diagnostic significance. Where the enzyme amylase is produced the starch grains are corroded or are partly or completely dissolved. The effect upon the wood-fiber cell walls of a decay-producing fungus is well illustrated in Plate 6. A tranverse section of the wood of *Ulmus americana* infected with a white rot fungus in which the fiber walls are remarkably reduced is shown at *A*, while *B* shows a transverse section of the normal uninfected wood of *Ulmus americana* with thick fiber walls.

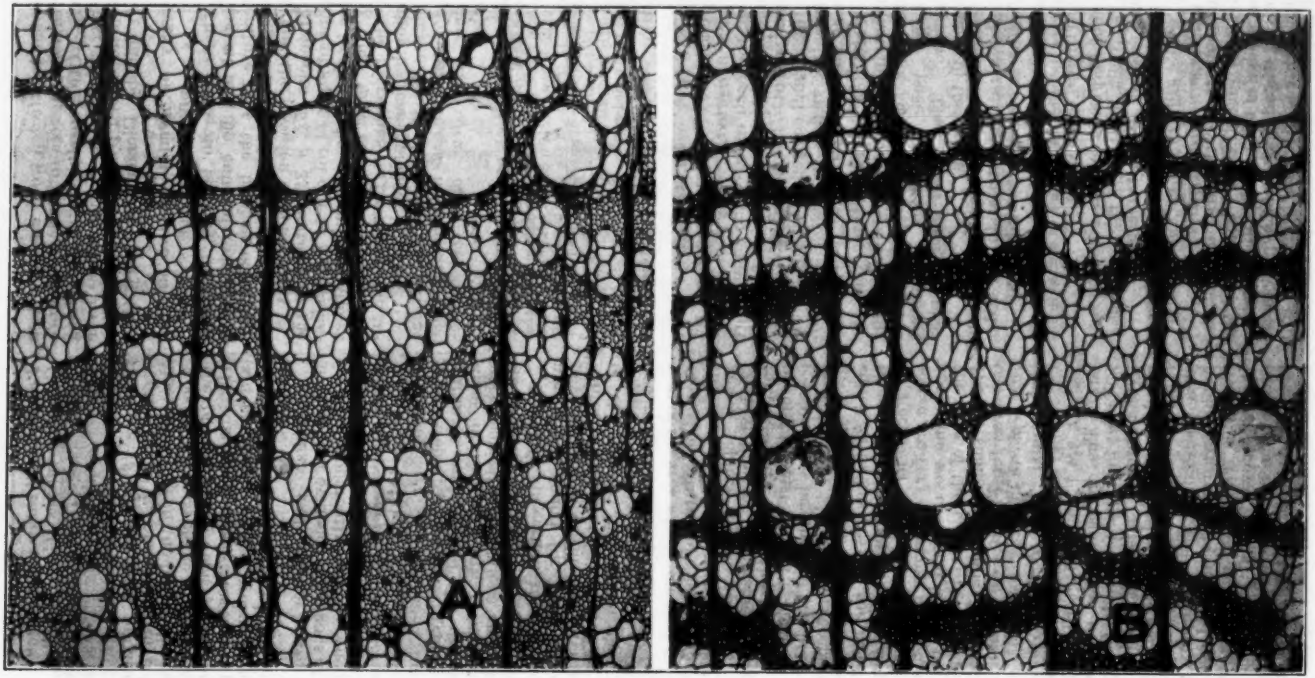
Color reactions produced in microscopical sections by different chemicals as indicators of the presence or absence of lignin or cellulose compounds in the infected tissues are useful in decay diagnosis and in a study of the microscopical characters of particular wood rots. Many such chemicals are given by Crocker (*18*). The writer has found the following of value also: Para-nitroaniline and hydrochloric acid, producing a bright orange yellow; pyrrole, producing a deep red; diphenylamine, producing an orange yellow; and both metol and hydroquinone, giving a light yellow color reaction.

For testing the presence of cellulose the iodine compounds giving violet to blue reactions are often helpful, also sulphuric acid and cuprammonia. A test for tannin is sometimes of value, as tannin is often found in decayed wood and absent in sound wood of the same species. Tubeuf (*79*, *p. 40*), using the ferric chloride test, found no reaction in sound spruce wood but secured a reaction for tannin in the same wood infected with dry rot produced by *Merulius lachrymans*. The standard stains used in microscopical technique serve to determine the constituents or the layers of the cell wall first attacked and whether dissolution is from within the cell outward or the reverse. The action of the fungus on the middle lamella as determined by staining is of particular value, as shown by the work of Spaulding (*77*).

It has been extremely difficult in the microscopical examination to demonstrate the presence of fungous hypae in the wood tissues. They are very small and quite transparent in the incipient stages of many rots, and are easily confused with hyaline fragments of the wood cells. The methyl violet Bismarck brown method for staining

EXPLANATORY LEGEND FOR PLATE 6

A.—Photomicrograph showing transverse section of *Ulmus americana* infected with a wood-destroying fungus. Note the thinness of the wood fiber cell walls. ×50. B.—Photomicrograph showing transverse section of sound *Ulmus americana*. The thickness of the normal wood fiber cell walls are very pronounced. ×50. (Courtesy of Mr. Koehler.)



(For explanatory legend see p. 538)

hyphae in infected wood has been used with some success in commercial work (*38*). Other useful stains (requiring longer preparation of the slides) have been developed by Sinnott and Bailey (*75*) and by Diemer and Gerry (*21*).

EXTENT OF HYPHAE IN AND BEYOND DISCOLORED AREAS

There seems to be very little histological and no cultural evidence in literature that the hyphae of certain wood-inhabiting fungi extend in and beyond the invasion discolorations.

Meinecke (*54, p. 28*) states that the incipient stage of rot due to *Echinodontium tinctorium* has a sound appearance but "has only recently been invaded by the mycelium." He terms the invading hyphae "pioneer hyphae."

Boyce (*8*) in describing the hyphae of *Polyporus amarus* in infected incense cedar (*Libocedrus decurrens*) states that—

> hyphae were commonly present in the apparently sound wood surrounding young pockets to a distance of 4 mm. (0.157 inch) and sparingly from that point to 8 mm. (0.314 inch) in a horizontal direction." In the case of the last (highest) pocket in a diseased tree the hyphae were abundant to a distance of 1.5 cm. (0.6 inch above the pocket) and sparingly from that point on to 7.8 cm. (3.07 inches), where they ended

Hiley (*35, p. 88*) in discussing the incipient rot produced by *Fomes annosus* in larch wood states that "stray hyphae were often found in the red rot region, and where hyphae could not be seen, the occurrence of the empty bore holes proved that they had formerly been present. Hyphae were even seen in the normal colored wood outside the turpentine region (bordering incipient decay and infiltrated with resin).

White (*89, p. 156*) states that the brown invasion zone noted in living trees attacked by *Fomes applanatus* "keeps pace with the advancing hyphae." This would imply hyphae present in this zone.

Schrenk (*68*) describing the mycelium of *Polyporus juniperinus* in red cedar (*Juniperus barbadensis*) states that

> the mycelium of the fungus is found in the wood between the holes (rot pockets), as well as in the sound (?) wood around the cavities,

and of *Polyporus carneus* Nees, he says

> the wood between the pockets has many hyphae, which pass from one pocket to another.

In his paper on the rot caused in white ash, *Fraxinus americana* L., by *Polyporus frazinophilus* Pk. (*71*) he finds that "the first hyphae are generally several rings" back from the outer edge of the invasion discoloration areas. Rhoads (*64*) found hyphae in the brown discolored wood (incipient rot) of *Lupinus arboreus* Sims. produced by *Polyporus ostreatus* (Jacq.) Que'l. and *Collybia velutipes* (Curt.) Que'l.

Münch (*58*) found hyphae of *Stereum purpureum* ahead of the brown invasion zone in infected poplar.

Boyce (*9*) in a recent publication states that "Apparently mycelium does not occur in the brown discolored wood in advance of the white spots" in the rot produced by *Fomes frazinophilus* (Pk.) Sacc. He also states that hyphae are not found in the brown invasion zone of the rot produced by *Fomes igniarius*.

Kauffman and Kerber (*43*) find hyphae and evidence of fungous attack in the apparently sound wood of *Robinia pseudo-acacia* attacked by (*Trametes robiniophila*) (*Polyporus robiniophilus* (Murr.) Lloyd).

MICROSCOPICAL EXAMINATIONS.—The extent to which the hyphae advance in and beyond the discolored areas of incipient rot, is shown by microscopical examinations, and is given in Table II.

Evidently, in certain rots the wood apparently sound for some distance beyond the edge of the discolored areas contains abundant hyphae. The face of the railroad tie section shown in Figure 3, A, when tested by culturing gave positive results from the greater part of the area which appeared entirely normal in texture and color. In a *Picea sitchensis* trunk split open for study the yellow spires, which indicate the farthest limit of the incipient stage of rot, extended up in the trunk 5 feet beyond the last signs of typical rot and for 30 inches beyond the last brownish discoloration. Hyphae were observed in a sample taken from the yellow spires 25 inches beyond the last faint brownish-colored areas. The hyphae of *Polyporus balsameus* in *Abies balsamea* are very numerous in the normal appearing wood several inches beyond the edge of the typical rot area (fig. 3 E). A somewhat similar condition is observed for *Trametes carnea* and *Lenzites sepiaria* in *Picea canadensis*. The longitudinal extent of the hyphae of these fungi beyond the discolored areas has not yet been satisfactorily worked out. The invasion zone of *Fomes igniarius* in *Populus tremuloides* shows hyphae present, although not in large numbers (fig. 3, B). In some collections of this rot showing a faint yellow area bordering the invasion zone hyphae are rarely found, and cultures from this region are usually negative. It is not known whether the hyphae are present and are merely difficult to distinguish or whether the discoloration has preceded the hyphae.

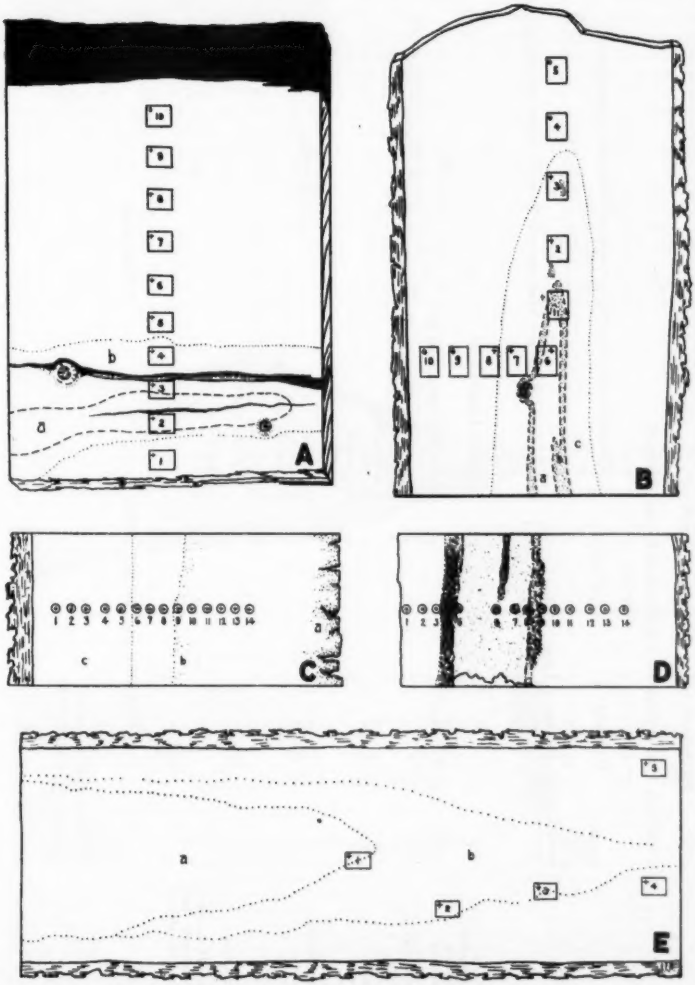


FIG. 3.—A.—Section of jack pine (*Pinus banksiana*) railroad tie treated with creosote showing areas where sections were taken for microscopical examination. Area *a* is the typical stage of brown rot produced by *Lentinus lepideus*, which apparently entered the tie after it was laid in the road bed. Area *b* represents the very faint yellowish-brown area surrounding the typical rot. The remaining area up to the creosoted portion is normal in color. Presence of hyphae is indicated by +, hyphae not observed by *O*. The distance from the edge of the plainly visible color in *A* to the center of No. 9 is 3$\frac{7}{8}$ inches. *B*.—Section of young tree of *Populus tremuloides* infected with *Fomes igniarius*. *a*, typical stage of rot; *b*, incipient *c*, very faint yellowish discoloration bordering on *b*. Hyphae were observed in 1 to 7 and in the margin of 8. From edge of area *b* to nearest edge of 8 is three-fourth inch. From edge of *b* to edge of *c*, longitudinally, is 2$\frac{1}{16}$ inches. C.—Section from living tree of *Abies balsamea* infected with *Polyporus balsameus*. Typical rot at *a*, incipient rot at *b*, with edge of faintest discoloration shown by dotted line. Sapwood region at *c* limited by straight dotted line. Cultures were made as indicated by numbers and positive results obtained from numbers 6 to 14, inclusive. Cultures from 6, 7, and 8, taken from apparently sound wood, are positive; cultures taken from the sapwood region are negative. No hyphae were observed in the sapwood. D.—Section of young tree of *P. tremuloides* infected with *F. igniarius*. Light shading represents typical rot, heavy shading the brown invasion zone. A zone line is shown at the bottom. Positive cultures were secured from the typical rot area and from one fragment taken from the invasion zone area. The areas showing no discoloration gave negative cultures. E.—Section from living tree of *Abies balsamea* infected with *P. balsameus*: *a*, buff yellow to brownish typical rot area, *b*, incipient faintly colored rot area. Sections for examination were cut as indicated. Hyphae were observed in all the sections and were fairly numerous in 4 and 5 which were taken from the normal colored wood. Distance from 1 in typical rot to 3 in incipient is 2$\frac{1}{4}$ inches. Distance from edge of *b* to center of 5 is 1$\frac{3}{4}$ inches.

TABLE II.—*Results of microscopical examinations of sections from various stages of rot and from apparently sound areas in wood infected by certain wood-destroying fungi*

[+ indicates hyphae observed; 0 indicates no hyphae observed]

Fungus	Host	Apparently sound; normal color	Incipient rot			Typical rot		Distance in inches from outer edge of discoloration to farthest advanced hyphae	
			Faint color	Discolored	Edge of pocket	Center of pocket	Badly rotted	Hyphae ahead of distinct discoloration	Hyphae within discoloration
Echinodontium tinctorium	*Tsuga heterophylla*	+	+	+			+	Radial 7/8, longitudinal	
Fomes applanatus	*Populus grandidentata*	+		+			+		
Fomes frazinophilus	*Fraxinus americana*	0	+	+			+	0	1/8 radial.
Fomes igniarius	*Populus tremuloides*	0	+	+			+	Radial 3/4, longitudinal 2 1/2	
Do	*Acer rubrum*	0	(a)	+			+	0	Up to edge of darker zones.
Fomes laricis	*Pseudotsuga taxifolia*	+	+	+			+		
Fomes pinicola	*Tsuga canadensis*	0	+	+			+	Radial 1 1/2, longitudinal	
Fomes roseus	*Pinus banksiana*	b +	+	+			+	Longitudinal	
Ganoderma tsugae	*Tsuga canadensis*	0	a +	+			+	Radial	
Lentinus lepideus	*Pinus banksiana*	+	+	+			+	3 1/2 radial	
Lenzites sepiaria	*Picea canadensis*	0	+	+	+	+	+	2 1/8 radial	
Pholiota adiposa	*Tilia americana*	0	+	+			+	0	Up to or nearly to edge.
Polyporus adustus (?)	*Liquidambar styraciflua*	0	+	+			+	0	Up to edge.
Polyporus anceps	*Picea canadensis*	0	+	+	+	+	+	0	Do.
Polyporus balsameus	*Abies balsamea*	+	+	+			+	Radial 1 3/4, longitudinal 12 1/2	
Polyporus ellisianus	*Pinus ponderosa*	0	+	+	+	+	+	0	Do.
Polyporus schweinitzii	*Picea sitchensis*	+	+	+			+	Radial 3 1/2, longitudinal 25 beyond pale brownish color.	
Polyporus sulphureus	*Pseudotsuga taxifolia*	0	+	+			+	0	Do.
Trametes carnea	*Picea canadensis*		+	+			+	Radial 2 1/8	
Trametes pini	*Pinus monticola, Pinus banksiana, Pseudotsuga taxifolia,* and *Picea canadensis.*	0	0	+	+	+	+	0	Do.

a Yellow brown. b Very faint color, pale greenish at edge. c Water-soak.

The presence of hyphae in the various areas in infected wood was determined by microscopical examination of radial and transverse sections specially stained for this purpose (figs. 3 and 4). The Bismarck brown-methyl violet method of staining (*38*) was used in the majority of cases. Plate 5 shows a radial section of *Picea sitchensis* containing minute hyaline hyphae of a wood destroyer, *Polyporus schweinitzii*, stained by the above method. The minute hyphae could not be observed in the wood cells before staining. The piece of wood from which this section was cut showed no discolorations to indicate the presence of a wood-destroying fungus.

Hyphae were noted in the apparently sound wood of *Pinus banksiana* infected with what appeared to be *Fomes roseus*. On close observation this area showed a very faint greenish color on the outer edges and a pale yellowish-brown color between this and the typical rot area.

Hyphae are present some distance beyond the discolored areas in wood infected with *Fomes laricis* and *Fomes pinicola*. The incipient decay produced in firs and hemlocks by the Indian paint fungus, *Echinodontium tinctorium*, is known to continue longitudinally about 4 to 6 feet ahead of the discolored areas. Microscopical examination discloses hyphae some distance radially from the faintest discolorations (fig. 4, B).

Microscopical evidence therefore indicates that in certain rots the hyphae advance as far as the edge of discoloration; and that in other rots, principally those of the brown rot group, the hyphae may be found a considerable distance beyond the faint discolored areas in the incipient rot region.

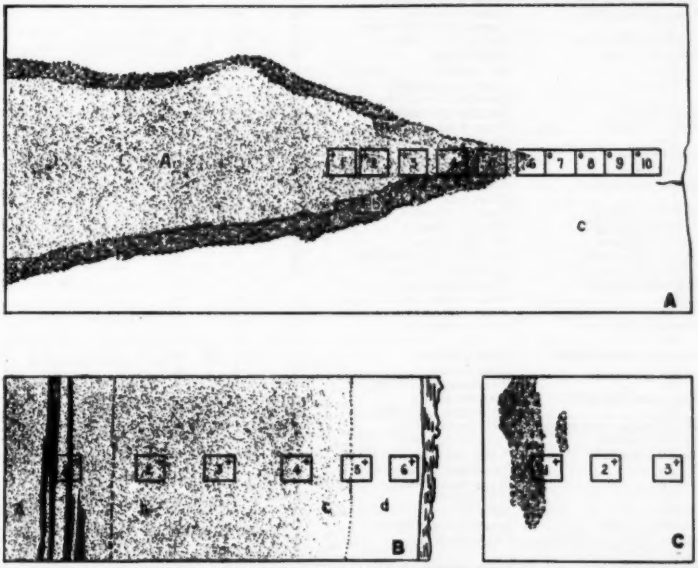


FIG. 4.—A.—Section of *Tilia americana* infected with *Pholiota adiposa*. *a*, incipient area; *b*, invasion zone; *c*, sound area. Sections for examination were taken from areas 1 to 10. Failure to find hyphae in 6 may have been due to the minuteness of the threads. No hyphae were observed in 7 to 10. Cultures made by using fragments from the invasion zone usually give positive results. B.—Section of fallen trunk of *Tsuga heterophylla* infected with *Echinodontium tinctorium*. Typical rot at *a* showing characteristic reddish streaks. Incipient rot at *b* with extremely faint discoloration at *c*. Apparently sound sapwood region at *d*. Hyphae were observed in all of sections 1 to 6. Apparently the fungus invaded the sapwood after the tree died. C.—Section of pulp log of *Picea canadensis* infected with *Lenzites sepiaria*. Typical rot in shaded areas. Very faint discoloration immediately surrounding the typical rot. Region near 2 and 3 apparently sound. Hyphae were observed in all the sections cut for examination. The distance from edge of typical rot to outer edge of 3 is 2⅛ inches.

CULTURAL EXPERIMENTS.—Fragments of infected wood were removed at regular intervals from the typical stages of rot, through the incipient stages and from the apparently sound wood beyond.[3] In the late typical stage of most of the brown rots it was very difficult to obtain cultures uncontaminated by molds. Apparently the shrinkage in this stage of decay

[3] The technique employed was essentially thatdescribed in U. S. Dept. Agr. Bul. 1262 : 40.

allows secondary fungi to enter more easily through the cracks. Table III shows the results of these cultural experiments. Figure 3, C and D, shows how the selection of inoculum fragments is made to extend from the typical decay to the sound areas. As a general rule the apparently sound regions beyond the incipient discolorations in the brown rots contain viable hyphae.

TABLE III.—*Distribution of hyphae of various fungi in infected wood as determined by various cultures*

[+ indicates positive cultures; 0 indicates negative cultures]

Fungus or rot	Host	Apparently sound; normal color	Incipient rot			Typical rot	
			Faint color	Discolored	Edge of pocket	Center of pocket	Badly rotted area
Ceratostomella	*Pinus strobus*	[a] +	+	+			
Echinodontium tinctorium	*Tsuga heterophylla*	+	+	+			+
Fomes applanatus	*Acer saccharum*	0	+	+			+
Do	*Populus grandidentata*	0	+	+			+
Fomes frazinophilus	*Frazinus americana*	0	+	+			+
Fomes igniarius	*Populus* sp	0	[b] 0	+			+
Do	*Acer rubrum*	0	0	+			+
Do	*Prunus emarginata*	0		+			+
Do	*Populus tremuloides*	0	0	+			+
Fomes laricis	*Pseudotsuga taxifolia*	0	+	+			+
Fomes pinicola	*Tsuga heterophylla*	0	+	+			+
Do	*Tsuga canadensis*	0	+	+			+
Fomes roseus	*Picea canadensis*	+	+	+			+
Do	*Pseudotsuga taxifolia*	+	+	+	+	+	+
Ganoderma tsugae	*Tsuga canadensis*	0	[a] +	+			+
Do	*Tsuga heterophylla*	0	+	+			+
Lentinus lepideus	*Pinus banksiana*	+	+	+			+
Lenzites sepiaria	*Picea canadensis*	0	+	+	+	+	+
Pholiota adiposa	*Tilia americana*	0	[d] +	[c] +			+
Polyporus adustus	*Liquidambar styraciflua*	0	+	+			+
Polyporus amarus	*Libocedrus decurrens*	0			+	+	
Polyporus anceps	*Picea canadensis*	0	+	+	+	+	
Do	*Pinus banksiana*	0	+	+			+
Polyporus balsameus	*Abies balsamea*	+	+	+			+
Polyporus ellisianus	*Pinus ponderosa*	0	+	+			+
Do	do	0	+	+		+	[f] +
Polyporus schweinitzii	*Picea sitchensis*	+	+	+			+
Do	*Larix decidua*	+	+	+			+
Do	*Pseudotsuga taxifolia*	+	+	+			+
Polyporus sulphureus	*Quercus* sp	0	+	[e] +			+
Do	*Pseudotsuga taxifolia*	0	+	+			+
Schizophyllum commune	*Betula papyrifera*	+	+	+			
Trametes carnea	*Picea canadensis*	+	+	+	+	+	+
Trametes pini	*Pinus banksiana*	0	0	+	+	+	+
Do	*Pseudotsuga taxifolia*	0	0	+	+	+	+
Do	*Pinus monticola*	0		+	+	+	
Do	*Pinus strobus*	0	+	+	+		+
Do	*Larix laricina*	0	+	+	+		+
Trametes serialis	*Pseudotsuga taxifolia*	0	+	+	+	+	
Brown butt rot; pocket rot.	*Thuja plicata*	0		+	+	+	+
Brown cubical rot	*Thuja occidentalis*	0	+	+	+	+	+

[a] White area adjacent to stained area. [b] Yellow. [c] Water-soak. [d] Red brown. [e] Black brown. [f] Rare. [g] Brownish.

Table III shows 8 of the fungi with positive cultures from the normal colored areas, 22 from the faint colored areas, 24 from the discolored areas, 9 from edge of pocket, and 24 from the typical rot areas. Only one case (*Polyporus ellisianus*) did not give cultures from the late typical stage of rot. The results with *Trametes pini* are interesting and indicate that hyphae do not develop beyond the outer edge of the incipient discolorations. This also appears to be true for *Polyporus anceps* in *Pinus ponderosa* and *Pinus banksiana*, and for *Polyporus circinatus* in *Picea canadensis*. The point to be emphasized here is that the hyphae of certain fungi, many of which produce brown rots, do extend some distance beyond the definitely discolored areas which are usually recognized as stages of incipient rot. The average distance to which these hyphae penetrate ahead of the discolorations in each case can be obtained only after many tests of freshly cut material.

THE EFFECT OF HYPHAE ON THE WOOD TISSUES

METHODS OF CELL WALL PENETRATIONS.—One of the earliest writers to figure the penetration of cell walls by fungous threads was Unger (*80*), who clearly showed the constriction of the hyphae in passing through the bore

hole. The first reference to wall penetration by wood-rotting fungi is given by Willkomm (*90*), who made several excellent drawings showing the hyphae in the wood tissues but showed no definite penetration of the hyphae through the cell walls. R. Hartig (*28, 29, 30*) was the first to demonstrate and illustrate correctly the penetration of cell walls by hyphae of wood-rotting fungi. Since Hartig's time comparatively little has been added to our knowledge of the subject. His work (*28*) shows that there are two principal methods of penetration exhibited by the various fungi studied. The (older) hyphae of the one group are always constricted when passing through the cell walls, while those of the other group show no constriction but pass freely through an opening in the cell wall as large or larger than the hyphal thread.

Hartig illustrates the larger hyphae of *Armillaria mellea* (*28, Pl. 11*) greatly constricted, with the small, apparently young hyphae showing no constriction. He shows the hyphae of *Polyporus borealis* markedly constricted (*28, Pl. 10*), with no enlargement near the host cell walls. But he figures the hyphae of *Polyporus fulvus* (*28, Pl. 7*) passing through the cell walls by way of large hourglass-shaped bore holes, and *Trametes pini* (*28, Pl. 6*), *Polyporus schweinitzii* (*28, Pl. 9*), and *Fomes igniarius* (*28, Pl. 16*) passing through cell walls without constriction. A few bore holes are shown with no hyphae passing through in *Stereum hirsutum* (*28, Pl. 18*). For *Fomes annosus* (*Trametes radiciperda*) a few bore holes and one indistinct penetration are noted (*28, Pl. 4*). No penetrations by hyphae are shown for *Polyporus sulphureus* (*28, Pl. 14*).

Lindroth (*45*) records the hyphae of *Polyporus nigricans, Polyporus betulinus,* and *Polyporus laevigatus* boring without constriction through the cell walls.

White (*89*) notes the constriction of hyphae of *Fomes applanatus* in infected wood. Hiley (*35*) records constriction of hyphae for *Dasyscypha calycina, Fomes annosus,* and *Polyporus schweinitzii* in larch wood. In the latter fungus the constriction is marked and in *Armillaria mellea* the hyphae are of the same size as the bore holes. These statements do not correspond with Hartig's records as given above.

Buller (*14, fig. 1*) illustrates the penetration of tracheid walls of pine by hyphae of *Lentinus lepideus.* No constriction is shown and the bore holes are larger than the hyphae.

The mechanics of penetration through plant cell walls of hyphae of various fungi have always interested pathologists. There are several possible ways. Openings already provided may be used (*36, 57, 72*), or by means of enzymic action (*3, 20, 26, 35, 42*) an opening may be dissolved out of the wall. A third possibility is the forcing of an opening by mechanical means (*31, 32, 52, 55*). Hawkins and Harvey (*32*) give a résumé of the literature on penetration of plant cell walls and conclude that some good evidence supports the view that certain parasites penetrate the hosts by mechanical means.

Many wood-inhabiting fungi pass from one cell to another through such natural openings as the simple or bordered pits. It is of interest to note that the hyphae of wood-destroying fungi as a rule do not seek these natural openings but pass through the cell walls at random, often passing through a large number of cell walls in a direct line, and often passing through the bordered pit very near its opening. In the case of wood-rotting fungi it would seem that the greater resistance offered by the thick, lignified walls of the tracheid and wood-fiber cells would preclude any possibility of a fungus hyphae forcing an opening by mechanical means only. The theory of enzyme action more nearly fits the facts observed.

SIZE AND SHAPE OF BORE HOLES.—The bore holes by which the hyphae pass through the cell walls vary considerably with the stage of the rot and with the fungus in question. In certain rots they are small in the incipient stage and become larger in diameter as the decay progresses (figs. 5 and 6).

In such rots as those produced by *Trametes pini,* where the hyphae are constricted, very little change in the diameters of the bore holes is noted (fig. 5, B; fig. 6, 1 to 12). Those through which the very young hyphae pass are rarely smaller in diameter than those through which the older and larger hyphae extend. Where constrictions occur in the hyphae when passing through the cell walls the bore holes are usually even, cylindrical, and perpendicular to the surface of the walls. This is brought out very well when the holes are seen in longitudinal section (fig. 5, Be and Fd).

In other rots the section view shows the bore holes with irregular outlines, in many cases resembling roughly an hourglass. This feature is always found in connection with bore holes which are usually twice to several times larger in diameter than the

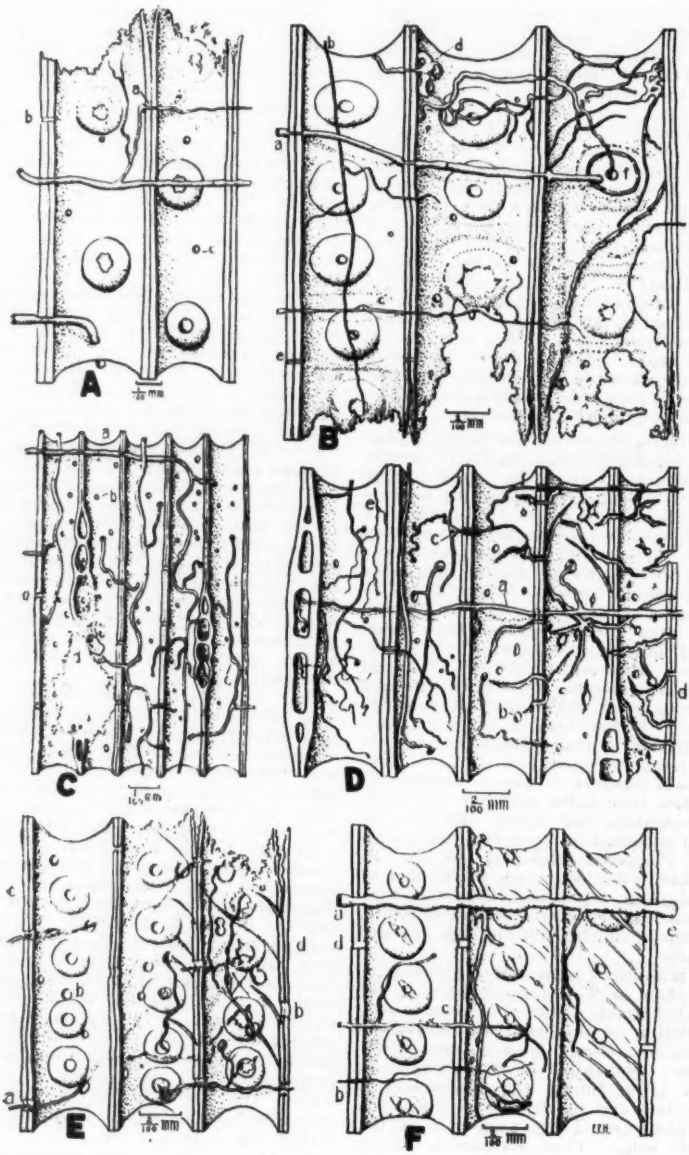


FIG. 5

FIG. 5.—A.—*Polyporus balsameus* in *Abies balsamea*. Typical stage of rot. Radial section through heartwood. *a*, Constriction of hyphae in passing through cell wall. *b*, *c*, bore holes produced by hyphae. B.—Radial section through heartwood of *Pinus strobus* infected with *Trametes pini*. Section taken near edge of white pocket. *a*, An older hypha of a brownish color showing the habit of constriction when passing through the cell walls; *b*, a young hypha, hyaline, and branched (the constriction of the thread does not appear in these young hyphae); *c*, characteristic clamp connection often found in wood infested by this fungus; *d*, tangled masses of apparently anastomosed hyphae; *e*, bore hole with fungus thread dropped out; *f*, embossed portion of bordered pit entirely decomposed. The effect on the middle lamella allows a separation of the individual cell walls. *C*.—Tangential section through the heartwood of *Tsuga heterophylla* infected with *Echinodontium tinctorium*. Section from typical stage of the rot. The general character of the dissolution of cell walls may be noted. *a*, an older hypha penetrating several cells walls and showing slight constrictions on passing through the bore holes; *b*, bore holes made by the hyphae in passing through the cell walls; *c*, characteristic clamp connection not often observed in the decayed wood; *d*, dissolution of the medullary ray and portions of the lignified cell wall of the tracheid. D.—Tangential section through the heartwood of *Picea canadensis* infected with *Trametes carnea*. Section taken from the edge of typical stage of the rot. *a*, hypha penetrating several cell walls and showing no constriction in passing through the walls. In this case the bore holes are usually much larger than the hyphae; *b*, characteristic bore hole with hyphal thread dropped out; *c*, this type and the simple type of clamp connection are frequently observed in the infected wood; *d*, peculiar uncommon form of shrinkage cracks found in the typical stage of rot. The spiral form of shrinkage crack is absent; *e*, very minute hyaline hyphae are often observed. The bore holes produced by these hypha are also larger than the hyphae. E.—Radial section through the heartwood of *Pinus banksiana* infected with *Lentinus lepideus*. Section from early typical stage of the rot. *a*, Hyphal thread penetrating tracheid cell wallse one of the very large and characteristic bore holes is shown at *b*, where the irregular surface of the bor; can be noted; *c*, a peculiar type of medallion or detour hypha commonly found in this material; *d*, spiral shrinkage cracks ascending from right to left and extending as far as the middle lamella of the cell wall. F.—Radial section through the heartwood of *Libocedrus decurrens* infected with *Polyporus amarus*. Section from the edge of the pocket of brown cubical rot. Hyphae are generally scarce in this rot. *a*. Large hyaline hypha penetrating several tracheid cell walls which show roughened surfaces due to the action of the fungus (the hypha is constricted in its passage through the walls); *b*, minute hyaline hyphae are occasionally observed and these appear to be slightly smaller than the bore holes they produce; *c*, hypha showing clamp connection; *d*, bore hole, showing the even smooth surface; *e*, spiral shrinkage cracks ascending from right to left are common in the typical stage of the rot and appear to be more numerous in the cells of the summer wood. The bordered pits show evidence of the fungus action and cases may be noted where little is left to indicate the original location of the bordered pit.

FIG. 6.—Penetration of hyphae of *Trametes pini* through tracheid cell walls of *Pinus strobus* is shown in 1 to 12. (The older, larger, brown hyphae in 1 diminish in size to the small hyaline hyphae shown in 10.) An oblique cut through hypha and bore hole, rather than the early stage of penetration, is shown in 11. Surface view of a hypha passing through a bore hole is shown in 12. The diameters of the bore holes vary but little. Radial section of *Pinus ponderosa* infected with *Polyporus anceps* (*P. ellisianus*) shown in 13. Incipient stage of rot, showing size of bore hole and constriction of the hyphae. The bore holes made by the very small hyphae seem to be larger than the hyphae. A spore-chain formation of the hyphae is often observed as shown in the drawing. Radial section of *Tilia americana* infected with *Pholiota adiposa* is shown in 14. Incipient stage of rot secured by inoculating a sterilized block with a pure culture of the fungus. The hyphae are slightly constricted in passing through the cell walls. Tracheid wall of *Picea sitchensis* infected with *Polyporus schweinitzii*, from the typical rot area, shown in 15, with penetration by hyphae and types of bore holes. Slight constriction of the hyphae in the larger threads (*d*). The bore holes are irregular and constricted at the middle lamella (*c* and *e*). Bore holes accompanying the very small hyphae seem larger than the threads passing through them. Radial section of *Populus balsamifera* infected with *Fomes igniarius* shown in 16. From the invasion zone area near the edge of the typical rot. *a*, One of the minute hyaline hyphae passing through bore holes in the cell walls of the wood fibers; *b*, one of the larger hyphae passing through the walls. The bore holes are very much larger than the hyphae; *c*, typical bore holes showing the greater resistance to dissolution exhibited by the middle lamella. Radial section of *Pinus banksiana* infected with *Lentinus lepideus* is shown in 17. From the faintly discolored area near edge of typical rot. Young hypha (*a*) and older hypha (*b*) passing through cell walls; *c*, type of bore hole showing constriction at middle lamella; *d*, characteristic "detour" or "medallion" hypha frequently found in this material. Radial section of *Betula papyrifera* infected with *Polyporus betulinus* is shown in 18. From typical rot area, showing penetration, size, and shape of bore holes. Radial section of *Betula papyrifera* infected with *Fomes fomentarius* is shown in 19. From near edge of typical rot area. The bore holes are of typical "hourglass" shape. Radial section of *Tsuga heterophylla* infected with *Echinodontium tinctorium* is shown in 20. From the early typical stage of rot. No constriction of the hyphae nor of the bore holes was observed. *a*, Older hypha with young branch at *b* showing penetration through the pit membrane; *c*, bordered pit showing action of the fungus in dissolving the embossed portion. Radial section of *Robinia pseudoacacia* L. infected with *Trametes robiniophila* (*P. robiniophilus*) shown in 21. From typical stage of rot, showing penetration of the tylose walls in the pore cells by the hypha. The bore holes are larger than the hypha. Semidiagramatic view of the bordered pit in the tracheid wall of *Pinus strobus* is shown in 22, with the hypha of *Trametes pini* passing through the wall at *a* and through the bordered pit at *b*. Older hypha (*a*) and young hypha (*b*) of *Trametes pini* passing through cell walls in *P. strobus* are shown in 23. Radial section of *Libocedrus decurrens* infected with *Polyporus amarus* is shown in 24. From edge of rot pocket. Penetration and characteristic constriction of older hypha and type of bore hole appear.

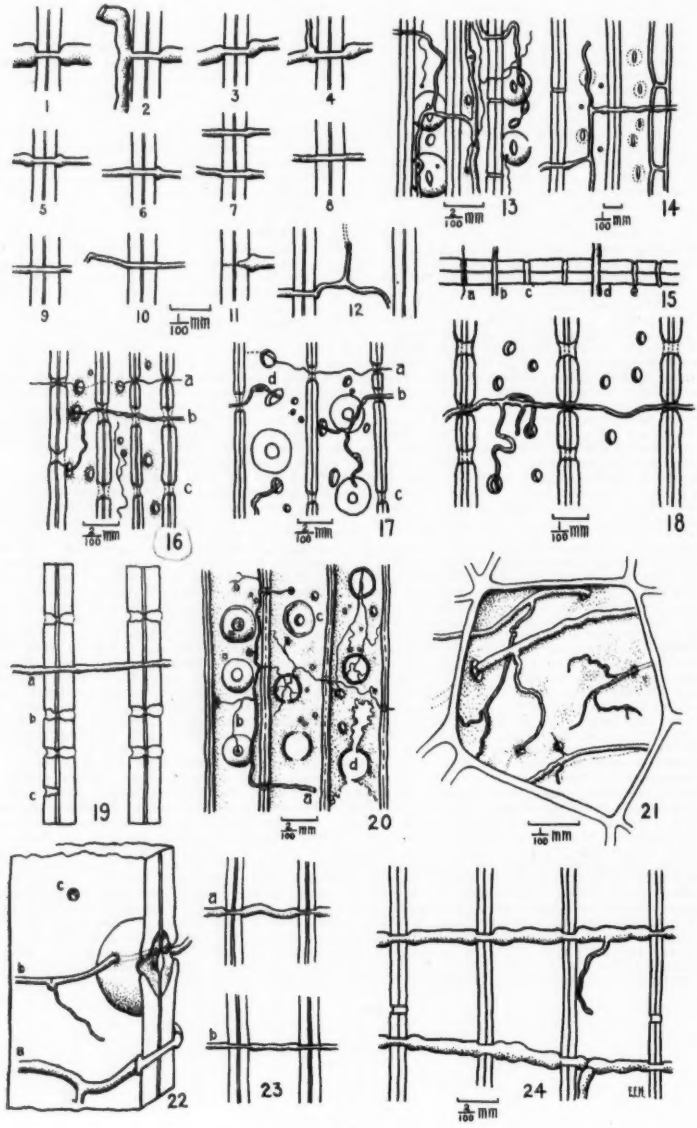


FIG. 6

(For explanatory legend see p. 547.)

hyphae which pass through them (16, 17, 18, 19, in fig. 6). The hourglass shape appears to be caused by the greater resistance of the middle lamella to the decomposition process (19b, fig. 6). In a few cases hyphae were noted passing through bore holes of the same diameter as the hyphae. This is often observed where young hyphae are penetrating the walls (8, 9, 10, in fig. 6). The hyphae pierce any part of the cell wall, often the curved or embossed part, or the membrane of the bordered pit; occasionally they pass through the pit openings (fig. 5, Bf and E).

Hiley (*35*) in discussing the hyphae in larch wood infected with *Fomes annosus* states that—

> to make these bore holes, the hyphal tips must excrete the necessary enzymes (presumably a lignase and a cytase). When once a hypha has gained passage from one tracheid to the next, the hole which it occupies ceases to enlarge, and it may reasonably be deduced from this that the enzymes are only secreted by the apices of the hyphae.

Observations on a number of fungi causing wood rot, and careful comparison of the very young hyphae with the older ones which showed constrictions, indicated that the bore holes were usually as large or only slightly larger in diameter than the diameter of the young hyphae found penetrating the cell walls. The relative size of bore holes in the tracheid cell walls in *Pinus strobus* infected with *Trametes pini* and of the hyphae producing them is shown in 1 to 10 of Figure 6. The bore hole produced by the largest hypha (fig. 6, 1) is little different in size from that produced by a very young hypha (fig. 6, 10), exhibiting no pronounced constriction. In (fig. 6, 11) is shown what might be taken for the early stage of penetration of a hypha, which is interpreted as the effect produced by sectioning obliquely through the hypha and the bore hole. Numerous hyaline hyphae smaller than those shown in 9 and 10 of Figure 6 were noted in the tracheids, but they were not observed penetrating the walls. Some idea of the number of bore holes in the infected tissues may be gained by the photomicrograph in Plate 7 showing *Trametes pini* in southern yellow pine. Apparently, the young hyphae can only dissolve the cell wall, and beyond a certain point are incapable of enlarging the bore holes. The hyphae continue to grow in diameter and the characteristic constrictions are produced where the hyphae pass through the bore holes in the cell walls. All the evidence appears to confirm Hiley's observations on *Fomes annosus*.

ABSENCE OF HYPHAE IN CERTAIN STAGES.—Very often when prepared sections, particularly those cut from the typical stage of a brown rot, are examined under the microscope, numerous bore holes and other evidence of fungus action appear but hyphae are absent. It is not clear whether absence of hyphae is due to loss during the handling of sections, or to resorption of the old hyphae, or, in the case of incipient stages of rot, to the invisibility of very fine threads (Pl. 5) (*43*).

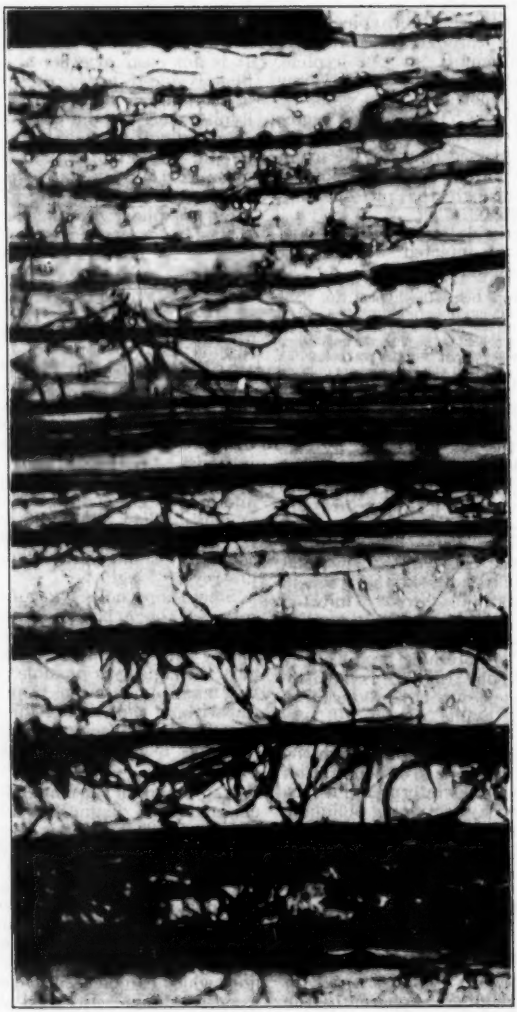
Buller (*11, 14*) states that in wood decayed by *Lentinus lepideus* the hyphae often disappear, leaving but a trace of their former existence in the form of hyphaelike deposits of calcium oxalate crystals.

Rhoads (*63*) finds scanty mycelium in the typical stage of rot produced by *Polystictus pargamenus*, and notes this peculiarity in other rots. He suggests that the enzymes produced by the fungus dissolve the older hyphae.

Kauffman and Kerber (*43*), working with the rot produced in black locust by *Trametes robiniophila*, had difficulty in finding hyphae of the fungus in the older stages of decay, though bore holes and other signs of fungus activity were evident. They find this a frequent phenomenon in the typical stages of rot caused by some of the common wood-destroying fungi.

Since bore holes vary for different rots (see above, p. 545), and certain infected woods exhibit bore holes considerably larger than the hyphae which pass through, it is possible that in cutting microtome sections from such material without embedding in celloidin or paraffin the hyphae may become broken and may eventually become lost from the section.

A summary of the main microscopical characters of diagnostic value assembled during this study is given in Table IV. In the absence of hyphae identifiable as wood destroyers the bore holes are perhaps of prime importance. Spiral cracks and corrosion marks are reliable when accompanied by hyphae, particularly when the hyphae show no characters such as buckles or medallions which can be used to class them as wood-rot fungi. In wood showing no distinctive gross or microscopical characters the proof that the hyphae observed belong to a wood-rot fungus rests entirely upon the success of cultural methods. (These observations may not hold for all species of wood and under all conditions. The number of species examined is limited, particularly for any given fungus, and the study should be continued to obtain further



The Diagnosis of Decay in Wood **PLATE 7**

Photomicrograph of a tangential section of southern yellow pine infected with *Trametes pini*. Note the numerous bore holes produced in the tracheid cell walls by the hyphae. ×250.

TABLE IV.—*Diagnostic characters of various fungi causing wood rots.—Observations based mainly on typical stage of rot*

Fungus	White rot	Brown rot	Zone lines	Bore holes large	Bore holes small	Spiral cracks	Old hyphae not constricted[a]	Old hyphae constricted[a]	Old hyphae with little or no constriction	Buckles noted	Medallion hyphae	Hyphae beyond incipient color
Armillaria mellea	×		×		×			×	×	0		
Echinodontium tinctorium		×	×	×			×			×		×
Fomes annosus	×				×			×		0		
Fomes applanatus	×		×		×			×				×
Fomes connatus	×		×	×			×			[b]×		
Fomes everhartii	×		×									
Fomes fomentarius	×		×	×			×			0		
Fomes fulvus		×	×	×		×	×					
Fomes frazinophilus	×		×	×			×			×		
Fomes igniarius	×		×	×			×			0		
Fomes laricis		×		×		×	×			×		×
Fomes nigrolimitatus	×		×		×				×	0		
Fomes pinicola		×		×		×	×			×		×
Fomes roseus		×		×		×	×			×		×
Ganoderma tsugae	×		×				×	[c]×	×	×		
Ganoderma curtisii	×		×							×		
Hydnum septentrionale	×		×	×			×			0		
Hymenochaete rubiginosa	×		×	[d]×	[c]×		×		[c]×	0		
Lentinus lepideus		×		×		×	×			×	×	×
Lenzites betulina		×		×		×	×					
Lenzites sepiaria		×		×		×	×			×	×	
Merulius lachrymans		×		×		×	×			×		
Pholiota adiposa		×			×			×		0		
Pleurotus ulmarius		×		×		×	×			0		
Pleurotus ostreatus	×			[b]×	[c]×		[f]×	[c]×		0		
Polyporus adustus (in *Populus* sp.)	×			×			×			×		
Polyporus amarus		×			×	×		×		×		
Polyporus anceps	×				×			×		×		
Polyporus balsameus		×		[c]×	×	×	[c]×	×		×		×
Polyporus berkeleyi		×		×			×			[b]×		
Polyporus betulinus		×		×		×	×			0		
Polyporus borealis	×				×			×		0		
Polyporus circinatus	×			×			×	[b]×		0		
Polyporus guttulatus		×		×		×	×			0		
Polyporus resinosus	×		×	×			×			0		
Polyporus robiniophilus	×		×	×			×			0		×
Polyporus schweinitzii		×			×	×		×	×	0		×
Polyporus squamosus	×			×			×	[b]×		×		
Polyporus sulphureus		×		×		×	×			×		
Polystictus abietinus	×		×	×			×			×		
Polystictus hirsutus	×			×			×			0		
Polystictus pargamenus	×		×	×			×			×		
Polystictus versicolor	×		×	×			×			0		
Poria incrassata		×	×	×		×	×			0		
Poria laevigata	×			×			×			0		
Poria subacida	×				×			×		×		
Stereum sulcatum	×		×		×			×		0		
Trametes carnea		×		×		×	×			×		×
Trametes malicola		×		×		×	×			0		
Trametes protracta		×		×		×	×					
Trametes serialis		×		×			×			×	×(?)	×
Trametes pini	×		×		×			×		×		
Trametes isabellina	×		×	×			×			0		
Xylaria polymorpha	×		×	[f]×	[c]×		[f]×	[c]×		0		

[a] Passage of hyphae through cell walls of host.
[b] Rare.
[c] In summer wood.
[d] In vessels.
[e] In fibers.
[f] Pitted tubes.
[g] Late typical stage.
[h] Only when passing through heavily lignified walls. (Buller.)

data.) Table IV shows that large holes are more commonly found in the brown rots than in the white rots (with a notable exception in *Polyporus schweinitzii*). The white pocket rots so far studied, as a rule show small bore holes and constricted hyphae. Spiral shrinkage cracks appear to be exclusive features of the brown rots and apparently the enzymes of the causal fungi have here played an important part. The fact that brown rot fungi can apparently remove from the cell wall much of the so-called cellulose complex, leaving most of the lignin compound (see under "Decay processes" above), may be a clue to the origin of these cracks. May not the cracks, forming at the weakest tension point, follow the hollows between the spiral thickenings laid down in the formation of the tracheid cell wall? Another question is suggested: Do shrinkage cracks appear only in the wood tissues infected by fungi or are they found in sound tissues as well? Upon this answer hinges the diagnostic value of shrinkage cracks in wood tissues where hyphae are not observed. It is not inconceivable that the enzymes of a wood-rotting fungus may affect the tissues ahead of the hyphae and so produce shrinkage cracks in tissues where hyphae are lacking(*19, 26, 35, 51, p. 379*).

As an example of the value of microscopical characters in decay diagnosis, the differences between *Trametes pini* and *Polyporus circinatus* may be cited. The hyphae of *Trametes pini* in *Picea canadensis* are constricted in passing through the comparatively small bore holes in the cell walls and are commonly seen to penetrate in a direct line across the section through many walls. Buckles are observed. The hyphae of *Polyporus circinatus* in *Picea mariana* are not constricted, and the bore holes are usually much larger than the hyphae passing through them. The majority of the hyphae run lengthwise of the long axis of the cell. Only a few penetrate many cell walls in a direct line, and none show buckles.

Again, in the incipient stage of *Echinodontium tinctorium* in *Tsuga heterophylla* bore holes and hyphae are numerous and the surfaces of the cell walls appear slightly pitted or corroded. The hyphae penetrate the tracheid cell wall and pass through the bordered pits, in places filling the cell cavities with masses of branched and twisted hyphae. The hyphae have a tendency to concentrate in the medullary ray cells. No great changes are noted in the cell walls In the typical stage there is a marked dissolution of the cell walls, particularly in the region of the ray cells (fig. 5, Cd.). The decrease in width of the cell walls is marked, the fungus attacking the tertiary wall first, the dissolution progressing toward the middle lamella. In most of the sections examined the middle lamella was apparently left unaffected. The entire cell wall was reduced to a width of 1.5 to 2.5 μ wide as compared to 2.5 to 3.2 μ wide in the normal wood. The walls show no evidence of shrinkage cracks, but grooves which can be distinguished are apparently caused by contact with hyphae on their inner surfaces. A reddish to brownish decomposition by-product is often found filling the tracheid and medullary ray cells.

Following are the results of microchemical tests made on sections of infected wood of *T. heterophylla* and *Abies grandis*. With Millon's reagent a reaction for proteins was obtained in and near the zone lines and the areas containing these by-products. In the typical stage of decay such reagents as chloroiodide of zinc, phloroglucin and hydrochloric acid, potassium permanganate, potassium dichromate, ferric chloride, aniline sulphate and paranitroaniline gave, in general, a reaction for the lignin compounds. The absence of tannin throughout the rotted area was also determined. In the bleached to white patches the reactions indicate that considerable of the cellulose compound is present. With tincture of alkanin slight traces of resin were found near the sapwood region. With iodine a few starch grains were observed in the less decomposed medullary rays.

CULTURAL CHARACTERS OF DECAY

The third step after examination for gross and microscopical characters of decay involves a cultural technique described in an earlier paper (*40*). Long and Harsch have shown (*50*) that cultural methods can aid in identifying the organism causing a specific decay in wood.[4] The cultural data on pure cultures of the fungus isolated from the infected wood and grown upon a suitable standard medium should include observations upon the source of inoculum; the macroscopic and microscopic characters of the mycelium, with emphasis upon secondary spore formation (*76*); the size, color, and character of the hymenial layers produced in the near-typical and typical pilei; and conditions of light and temperature to which the cultures were exposed. To these data may be added proof of the important

[4] An important paper by C. W. Fritz (*25*) has come to the writer's attention since the preparation of the present papers.

question whether the isolated fungus, when placed in contact with sterilized sound wood under favorable conditions, produces a rot identical with that from which it was isolated.

The one difficulty to be guarded against is that of contaminations. Contaminating fungi present in the sample produce mixed cultures of very uncertain value; and the contaminations incidental to the technique of culturing are usually due to fungi which either inhibit or mask the development of wood destroyers on the agar. Mention has already been made of the fact that the late typical stage of the brown rots often shows a large amount of mold contamination when cultured. For critical study such material is obviously objectionable.

For extensive comparison of the color reaction produced by specific fungi, Long and Harsch (*50*) have recommended various media, but in routine commercial diagnosis the use of 8 or 10 different media is impracticable. Plain malt agar slanted in tubes has been found a very satisfactory standard medium in the present cultural work.

ARTIFICIAL PRODUCTION OF TYPICAL DECAYS

White (*89*) has noted the fact that there appears to be one general weakness in method throughout the works on forest pathology. This weakness is the identification of causal agents of wood rots based almost entirely upon the more or less constant association of the fungus with the rotted wood. In plant pathological investigations and in all standard bacteriological research Koch's rules are applied in their entirety in order thoroughly to establish the relationship between the causal organism and the symptoms. The comparison between bacteriological technique and organisms involved and the technique and organisms involved in wood-rot studies can not be drawn too closely. However, in the study of wood rots only the first rule, that of constant association of the organism with the disease, has been applied with any degree of consistency. It is equally important to establish with precision the relation between cause and effect in the study of wood rots as it is in the study of other plant diseases. The fact that more than one organism may be found developing simultaneously in the same substratum is sufficient ground for establishing a more accurate test for the identification of a wood-destroying organism with the particular type of decomposition produced in wood.

Only a few of the papers examined give cultural data showing the relationship between fungus and wood rot. Of these, only the work on *Fomes applanatus* by White (*89*) indicates intentional application of the rules of proof. He inoculated basswood, poplar, pine, spruce, tamarack, hemlock, fir, cedars, maple, and willow blocks with pure cultures of *Fomes applanatus* and secured typical rots in all but the conifers, where slight infection only was noted. Typical sporophores shedding mature spores were produced.

Abbott (*1*) secured the typical decay caused by *Trametes pini* in tamarack, pine, hemlock, spruce, balsam, birch, and oak by inoculation with pure cultures of the organism. The above order of the hosts, according to Abbott, is the order of susceptibility to the fungus.

Bayliss (*4*) produced the typical rot caused by *Polystictus versicolor* Fr. by inoculating various hardwoods with pure cultures of this organism. Typical sporophores were also produced, partly under natural conditions, on the infected blocks. Infection was not secured in the wood of pine and larch.

Zeller (*92*) used *Lenzites sepiaria* to inoculate blocks of southern yellow pine for durability tests and noted that the typical rot was produced in all cases.

The writer has produced incipient and typical decay with a number of wood-rotting fungi developing under artificial conditions. The following method was used in conducting these experiments:

(1) Numerous field observations (extending over several years) were assembled to check the constant association of the organism with the particular wood rot in the typical stage. (2) The organism was isolated from the decayed wood and pure cultures established, using fragments of decayed wood, sporophore tissue, and spores. (3) Sound sterilized wood was inoculated with pure culture inoculum, to prove that the organism can produce the incipient and typical stages of decay. (4) The organism was reisolated from the inoculated wood and compared with the original pure cultures or stock cultures, or with both. In the isolation of the organism from the piece of rotted wood 10 to 12 tubes were prepared from each wood sample (fig. 3, A). The fragments were selected from various areas on the surface representing both incipient and typical stages of decay. Subcultures were then made from these original cultures. Two sizes of sound wood pieces were used, each an inch square, the one being 5½ inches long, the other 11

inches long. Special length test tubes were used with the 11-inch sticks. A plug of cotton 2 inches long was placed in the bottom of each tube, the stick was next inserted and 200 c. c. of distilled water poured in. The tubes were then autoclaved for 45 minutes at 15 pounds steam pressure. The sticks were next inoculated by placing a small fragment of the mycelial growth taken from a pure culture of known fungus upon the stick at a point approximately 5 inches up from the base.

In some cases the inoculum used was from pure cultures secured from spores or from sporophore tissue of the fungus. A piece of waxed paper was next placed over the cotton plug and fastened closely to the tube by means of a strip of heavy gummed paper. Most of the tubes were kept 8 to 12 months or longer before they were opened and the wood examined for results. In a few cases, 6 months or less produced the typical stage of decay in the portion of the stick where the moisture conditions were most favorable to the action of the fungus. At the end of the test, blocks were cut from the test sticks and cultures made from these in the usual manner (*40*). These were carefully compared with the pure cultures previously obtained and with stock cultures of known fungi secured from spores or from sporophore tissues. The results of the tests are given in Table V. Some of the fungi were inoculated on various hosts in order to secure their reactions on each. Of the 29 fungi used in the tests, 2 produced perfect sporophores and 4 produced poroid growths on the infected wood. *Xylaria polymorpha* produced sterile, black, club-shaped growths.

In several cases the preference for the sapwood over the heartwood was quite pronounced and the organism developed rapidly and extensively on the sapwood blocks but barely covered the surfaces of the heartwood blocks (Pl. 9, C and D). The effect upon the wood was found to be in somewhat the same relationship. For instance, *Polyporus adustus* (?), *Fomes igniarius*, *Fomes applanatus*, *Polyporus anceps*, and *Polystictus versicolor* were inoculated on both sapwood and heartwood blocks and all five appeared to develop more rapidly upon the sapwood.

The results secured by inoculating *Tilia americana* with *Xylaria polymorpha* were very striking. The typical white spongy-rot accompanied by a narrow black zone line formed across one end of the block (Pl. 11, A) was disclosed upon splitting open the block. The surface of the block and the cotton in the bottom of the tube were covered in spots with grayish-black mycelial wefts and with the thin black crustlike layers so typical of the roots of *Acer saccharum* infected with this fungus. Sterile fruiting bodies also appeared at the base of the blocks.

The typical brown cubical rots of *Lenzites sepiaria* and of *Polyporus schweinitzii*, charcoal-like in consistency, were produced artificially in blocks of *Picea sitchensis* (Pl. 11, B and C) and of *Lentinus lepideus*, *Fomes laricis*, *Fomes roseus*, and *Fomes pinicola* (Pl. 8, A to H). The rot produced by *Lenzites sepiaria* showed a greater tendency to form numerous shrinkage cracks which divided the rotted wood into small cubes.

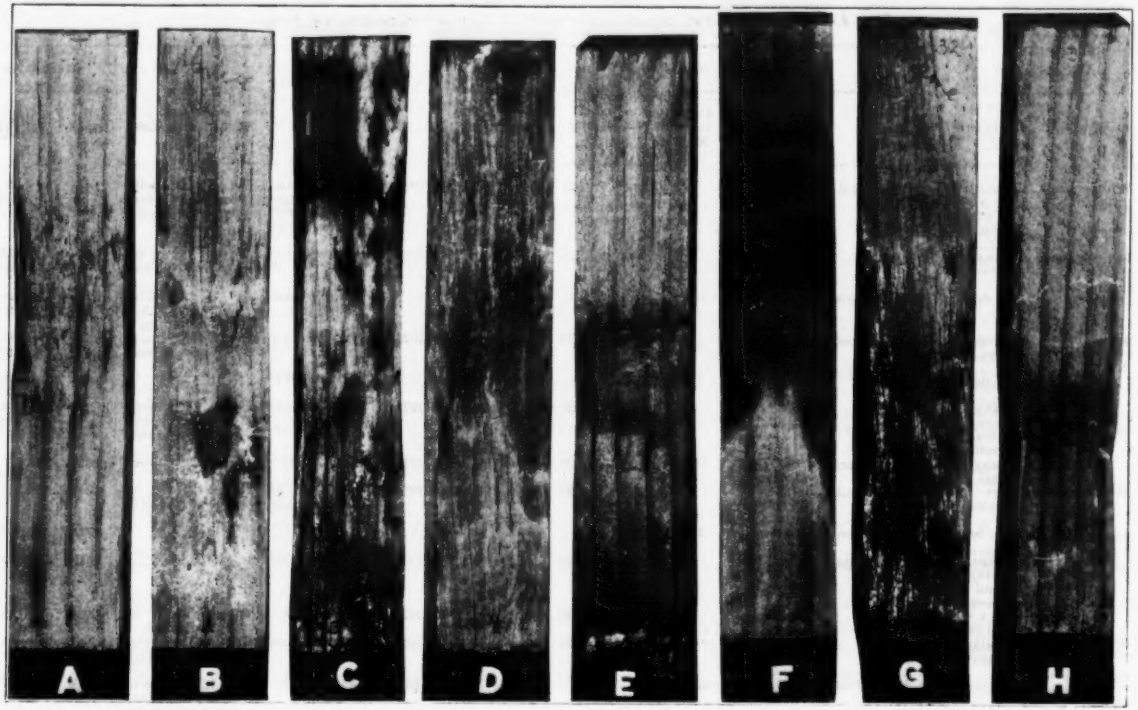
The rot produced artificially in *Picea sitchensis* and *Tsuga heterophylla* by *Trametes pini* is quite typical (Pl. 11, D). White pockets are numerous and typical narrow brownish zone lines are formed across and close to the ends of the pieces. The wood surrounding the pockets is stained reddish to reddish brown.

A valuable cultural character (which unfortunately does not always develop) is the production in pure cultures of near-typical to typical sporophores from which mature spores are often cast (Pl. 10). The sporophores, though diminutive, are frequently characteristic enough for identification and the spore casts furnish a means of checking the purity of the culture, as well as the character of the spores.

A cultural method using sound sterilized wood as a medium and in vitro applicable to the study of unidentified wood rots will be found helpful in verifying the identity of causal fungi in regions where the rots are numerous and where the sporophores rarely develop. In the younger stands of timber old enough to contain rot but too young to bear sporophores of the rot fungi, this type of cultural test will serve as a valuable check of the data obtained from a study of the gross, microscopical, and cultural characters.

EXPLANATORY LEGEND FOR PLATE 8

Brown rots produced by inoculation, showing typical rot with characteristic shrinkage in the rotted areas A and B.—*Fomes laricis*, taken from *Pseudotsuga taxifolia*, on *Picea sitchensis*. C.—*Lenzites sepiaria* on *Picea sitchensis*. D.—*Fomes roseus*, taken from *Picea canadensis*, on *Pinus strobus*. E.—*Lentinus lepideus*, taken from *Pinus taeda*, on *Picea sitchensis*. F.—*Fomes laricis* on *Pinus strobus*. G.—*Trametes carnea* on *Pinus strobus*. H.—*Fomes pinicola*, from *Tsuga heterophylla*, on *Picea sitchensis*.—Eight-elevenths natural size.



(For explanatory legend see p. 554)

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TABLE V.—*Results of cultural experiments with certain wood-destroying fungi*

Fungus	Association		Isolation—Pure cultures				Inoculation				Reisolation	
	Symptoms	Hosts	From—	Host	Culture No.	Date	Culture No.	Date	Medium	Date and results	Culture No.	Date and results
Fomes applanatus.	White mottled rot.	Many hardwoods, *Tsuga heterophylla.*	Typical rot.	*Acer saccharum.*	148	Nov. 7, 1921	148	Mar. 17, 1923	*Populus tremuloides;* sapwood+H_2O, heartwood+H_2O.	Oct. 1, 1923; incipient and typical rot, zone lines.	148a	Oct. 18, 1923; positive.
Fomes igniarius.	White spongy rot.	Many hardwoods.	do	*Populus balsamifera.*	154	Nov. 14, 1921	154	Dec. 15, 1922	*Pinus strobus*+H_2O.	May 15, 1923; incipient rot and zone lines.	154a	May 30, 1923; positive.
Do	do	do	Sporophore tissue.	do	154A	Dec. 6, 1921	154A	do	*Populus tremuloides*+H_2O.	Oct. 1, 1923; incipient and typical rot, zone lines.	154b	Oct. 18, 1923; positive.
Fomes laricis.	Brown cubical rot.	Several conifers.	Typical rot.	*Pseudotsuga taxifolia.*	268	Mar. 17, 1923	268	Mar. 17, 1923	*Picea sitchensis*+H_2O.	Oct. 1, 1923; incipient and typical rot.	268a	Oct. 20, 1923; positive.
Do	do	do	do	do	268	do	268	do	*Pinus strobus*+H_2O.	do	268b	Oct. 23, 1923; positive.
Fomes pinicola	do	Many conifers and *Alnus tenuifolia.*	do	*Tsuga heterophylla.*	31	Aug. 16, 1920	31	Mar. 14, 1923	*Picea sitchensis*+agar.	Oct. 1, 1923; incipient and typical rot; poroid growths.	31a	Oct. 16, 1923; positive.
Fomes roseus	do	Many conifers.	Sporophore	*Picea canadensis.*	*6364	Dec. 15, 1921	6364	Mar. 10, 1921	Spruce+H_2O	Feb. 1922; typical rot.	6364a	May 7, 1922; positive.
Ganoderma curtisii.	White pocket rot.	*Acer negundo.*	Typical rot.	*Acer negundo.*	134	Aug. 29, 1921	134	Apr. 14, 1923	*Pseudotsuga taxifolia*+agar.	Oct. 24, 1923; incipient and typical rot.	134a	Jan. 8, 1924; positive.
Do	do	do	Sporophore.	do	134A	do	134A	do	do	do	134b	Jan. 10, 1924; positive.
Ganoderma tsugae.	do	*Tsuga heterophylla* and *Tsuga canadensis.*	Typical rot.	*Tsuga canadensis.*	10F	Nov. 14, 1921	10F	Mar. 14, 1923	*Picea sitchensis*+H_2O.	do	10F	Oct. 31, 1923; positive.
Hymenochaete rubiginosa.	do	Several hardwoods.	do	*Castenea dentata.*	273	Apr. 12, 1923	273	Apr. 14, 1923	*Populus tremuloides.*	Oct. 1, 1923; incipient stage.	273a	Oct. 18, 1923; positive.
Lentinus lepideus.	Brown cubical rot.	Conifers and hardwoods.	Spores	From sporophore *P. taeda.*	*6285	Dec. 15, 1921	6285	Mar. 10, 1921	*Picea sitchensis*+H_2O.	Typical rot and hornlike growths.	6285a	June 6, 1922; positive.
Do	do	do	Typical rot.	*P. taeda*	*6285A	do	6285A	Mar. 14, 1923	*Pinus strobus*+H_2O.	Oct. 1, 1923; incipient and typical rot; badly rotted.	6285b	Oct. 17, 1923; positive.

Lenzites sepiaria.	....do....	Many conifers and *Alnus tenuifolia.*	Sporophore.	*Picea canadensis.*	63	Mar. 4, 1921	63	Dec 15, 1921	*Picea sitchensis* +H_2O.	Jan. 6, 1922; typical rot.	63a	Jan. 6, 1923; positive.
Pholiota adiposa.	Brown mottled rot.	*Abies grandis, Tilia americana,* and other hardwoods.	Typical rot.	Sporophore from *Tilia americana.*	243	Jan. 10, 1922	243	Jan. 30, 1922	*Tilia americana* +agar.	Incipient and typical rot; typical sporophores.	248	Dec. 11, 1922; positive.
Do	....do....	....do....	Spores	....do....	18K	Dec. 18, 1921	18K	Jan. 10, 1922	*Tilia americana* +H_2O.	Mar. 3, 1923; incipient and typical rot.	18K	Mar. 15, 1923; positive.
Polyporus adustus (?).	White spongy rot.	*Liquidambar styraciflua.*	Typical rot.	*Liquidambar styraciflua.*	138	Oct. 11, 1921	138	Mar. 17, 1923	*Populus tremuloides;* heartwood+H_2O.	May 15, 1923; typical rot and zone lines.	138a	May 31, 1923 positive.
Do	....do....	....do....	....do....	....do....	138	....do....	138	....do....	*Populus tremuloides;* sapwood+H_2O.	Oct. 1, 1923; incipient and typical rot zone lines.	138b	Oct. 17, 1923 positive.
Polyporus amarus.	Brown pocket rot.	*Libocedrus decurrens.*	....do....	*L. decurrens.*	106	June 29, 1921	106	Dec. 15, 1922	*Picea sitchensis* +H_2O.	May 15, 1923; incipient discoloration; no typical rot.	106a	Oct. 10, 1923; positive.
Polyporus anceps.	White pocket rot.	*Pinus ponderosa* and *Picea canadensis.*	Incipient rot.	*Picea canadensis.*	270	Jan. 30, 1921	270	....do....	*Picea canadensis*+H_2O.	May 15, 1923; typical rot and poroid growths shedding spores.	270a	May 26, 1923 positive.
Do	....do....	*Picea canadensis.*	Typical rot.	....do....	270A	....do....	270A	Mar. 14, 1923	*Pinus strobus* + agar.	Oct. 1, 1923; incipient rot.	270c	Oct. 19, 1923; positive.
Polyporus anceps, 3 tubes.	....do....	....do....	....do....	....do....	270A	....do....	270A	....do....	*Pinus ponderosa;* sap and heartwood +H_2O.	Oct. 1, 1923; incipient rot in heartwood; typical rot in sapwood; poroid growths.	270b	Oct. 15, 1923; positive.
Polyporus balsameus.	Brown cubical rot.	*Abies balsamea* and *Thuja occidentalis.*	....do....	*Abies balsamea.*	230	Oct. 13, 1922	230	Dec. 15, 1922	*Picea canadensis*+H_2O.	May 15, 1923; incipient and typical rot.	230a	May 31, 1923; positive.
Polyporus borealis.	White mottled rot.	*Picea engelmanii, Picea canadensis,* and *Acer saccharum.*	....do....	*Acer saccharum.*	244	Jan. 10, 1922	244	Apr. 14, 1923	*Picea sitchensis* +agar.	Oct. 1, 1923; incipient rot.	244a	Oct. 15, 1923; positive.
Polyporus elisianus.	White pocket rot.	*Pinus ponderosa.*	....do....	*Pinus ponderosa.*	269	Jan. 30, 1921	269	Dec. 15, 1922	....do....	May 15, 1923; typical rot and poroid growths.	269a	May 31, 1923; positive.
Do	....do....	....do....	....do....	....do....	269	....do....	269	....do....	*Picea sitchensis* +H_2O.	Oct. 1, 1923; incipient and typical rot.	269b	Oct. 12, 1923; positive.

[a] Pure cultures secured from Dr. C. J. Humphrey.

TABLE V.—*Results of cultural experiments with certain wood-destroying fungi*—Continued

Fungus	Association		Isolation—Pure cultures				Inoculation				Reisolation	
	Symptoms	Hosts	From—	Host	Culture No.	Date	Culture No.	Date	Medium	Date and results	Culture No.	Date and results
Polyporus pilotae.	White pocket rot.	*Castanea* and *Quercus* spp.	Typical rot.	*Castanea dentata.*	222	July 31, 1922	222	Mar. 14, 1923	*Picea sitchensis* +H_2O.	Oct. 1, 1923; incipient rot.	222a	Oct. 13, 1923; positive.
Polyporus schweinitzii.	Brown cubical rot.	Many conifers.	do	*Larix decidua*	133	Aug. 27, 1921	133	Jan. 9, 1922	*Picea sitchensis* +agar.	Oct. 26, 1922; typical rot.	133a	Oct. 30, 1922; positive.
Do	do	do	do	do	133	Aug. 28, 1921	133	Dec. 15, 1921	*Pinus strobus*, heartwood.	May 15, 1923; incipient rot.	133b	May 31, 1923; positive.
Do	do	do	Sporophore tissue.	do	133A	do	133A	do	Spruce+H_2O	Mar. 15, 1923; incipient; Oct. 1, 1923, typical.	133c	Oct. 12, 1923; positive.
Polyporus stipticus.	White pocket rot.	*Pinus ponderosa.*	Incipient rot.	*Pinus ponderosa.*	121	Aug. 17, 1921	121	Apr. 3, 1923	*Picea canadensis*+H_2O.	May 15, 1923; typical rot and poroid growths.	121a	Aug. 6, 1923 positive
Polyporus sulphureus.	Brown cubical rot.	Several conifers and *Quercus* sp.	Typical rot.	Red oak	157	Nov. 17, 1921	157	Dec. 15, 1922	*Picea sitchensis*+H_2O.	May 15, 1923; incipient and typical rot.	157a	July 6, 1923; positive.
Do	do	Conifers and hardwoods.	Incipient rot.	do	157A	do	157A	Mar. 11, 1922	Spruce+H_2O	Nov. 18, 1922; incipient and typical rot.	157b	Dec. 16, 1922; positive.
Polystictus versicolor.	White spongy rot.	Many hardwoods.	Typical rot.	*Ulmus americana.*	255	Feb. 24, 1923	255	Mar. 17, 1923	*Populus tremuloides;* sapwood+H_2O.	May 17, 1923; typical rot.	255a	July 15, 1923; positive.
Pleurotus ostreatus	do	*Ulmus americana*	do	*Populus grandidentata.*	191	Nov. 17, 1921	191	Dec. 15, 1922	*Populus tremuloides* +H_2O	Jan. 6, 1923; typical rot and dimidiate sporophores.	192a	Feb. 12, 1923; positive.
Do	do	*Populus tremuloides.*	Spores	do	192	do	192	do	do	do	192b	Do.
Do	do	*Populus grandidentata* and other hardwoods.	Sporophore tissue.	do	192A	do	192A	do	do	do	192c	Do.
Schizophyllum commune.	White rot	Many hardwoods.	Typical rot and spores.	*Betula papyrifera.*	258	Jan. 10, 1923	258	Mar. 14, 1923	*Pinus strobus* +*agar.*	Oct. 1, 1923; incipient rot and faint typical rot.	258a	Oct. 18, 1923; positive.

Stereum sulcatum.	White pocket rot.	Several conifers.	Typical rot.	*Larix occidentalis.*	33	Aug. 25, 1920	33	....do....	*Pinus strobus* +H_2O.	Jan. 8, 1924; typical rot.	33a	Feb. 16, 1924; positive.
Do	....do....	....do....	....do....	....do....	33	....do....	33	....do....	*Picea sitchensis*+H_2O.	Oct. 1, 1923; incipient rot.	33b	Oct. 31, 1923; positive.
Tramentes carnea.	Brown cubical rot.	Many conifers.	Incipient rot.	*Picea canadensis.*	74	Mar. 18, 1921	74	Dec. 15, 1921	Spruce+H_2O...	Oct. 26, 1922; typical rot and poroid growths.	74a	Dec. 20, 1922; positive.
Do	....do....	....do....	Sporophore tissue.	....do....	74A	....do....	74A	Mar. 14, 1923	*Pinus strobus* +H_2O.	Oct. 1, 1923; incipient and typical rot.	74b	Oct. 15, 1923 positive.
Trametes pini.	White pocket rot.	....do....	Typical rot.	....do....	73	Mar. 8, 1921	73	Dec. 15, 1921	Spruce+H_2O...	Oct. 26, 1922; typical rot.	73a	Jan. 14, 1923; positive.
Do	....do....	....do....	Spores....	From sporophores.	[a] 4580	Dec. 15, 1919	4580	Mar. 10, 1921	....do....	Typical rot....	4580a	July 10, 1922; positive.
Trametes serialis.	Brown cubical rot.	Conifers; common *Pseudotsuga taxifolia.*	Typical rot.	*Pseudotsuga taxifolia.*	37	Oct. 19, 1920	37	Dec. 15, 1922	*Picea sitchensis*+H_2O.	Oct. 1, 1923; incipient and typical rot and poroid growths.	37a	Oct. 19, 1923; positive.
Xylaria polymorpha.	White spongy rot, black zone lines.	Maple, basswood, oak.	....do....	*Acer saccharum.*	146	Jan. 4, 1922	146	Jan. 30, 1922	Basswood+ agar.	Nov. 18, 1922; typical rot.	246	Dec. 12, 1922; positive.

[a] Pure cultures secured from Dr. C. J. Humphrey.

DISCUSSION

The methods employed in diagnosing decay in wood have been presented with sufficient detail to show that many of the questions on wood decay asked by both scientist and lumberman can be accurately answered. Proving that decay is present or absent, and that the causal organism is alive or dead, and whether the fungus is a wood destroyer or a staining organism, is relatively easy in a majority of cases. This has been demonstrated by the diagnosis of over 1,500 samples sent to the laboratory for examination during two years. Gross, microsopical, and cultural characters were used in most of the diagnoses. A study of the microsocopical characters is made easier by a collection of microscopical mounts showing the characters of normal sound wood of various species. A similar collection of wood decayed by known fungi can be used to advantage in comparisons made with sections cut from the unidentified sample. A collection of stock pure cultures of the numerous fungi producing decay in wood is indispensible to the successful application of the cultural methods of diagnosis. The way in which cultural characters may furnish the evidence necessary to establish identity of the causal fungus, in addition to macroscopical and microscopical data, is well illustrated by the following examples:

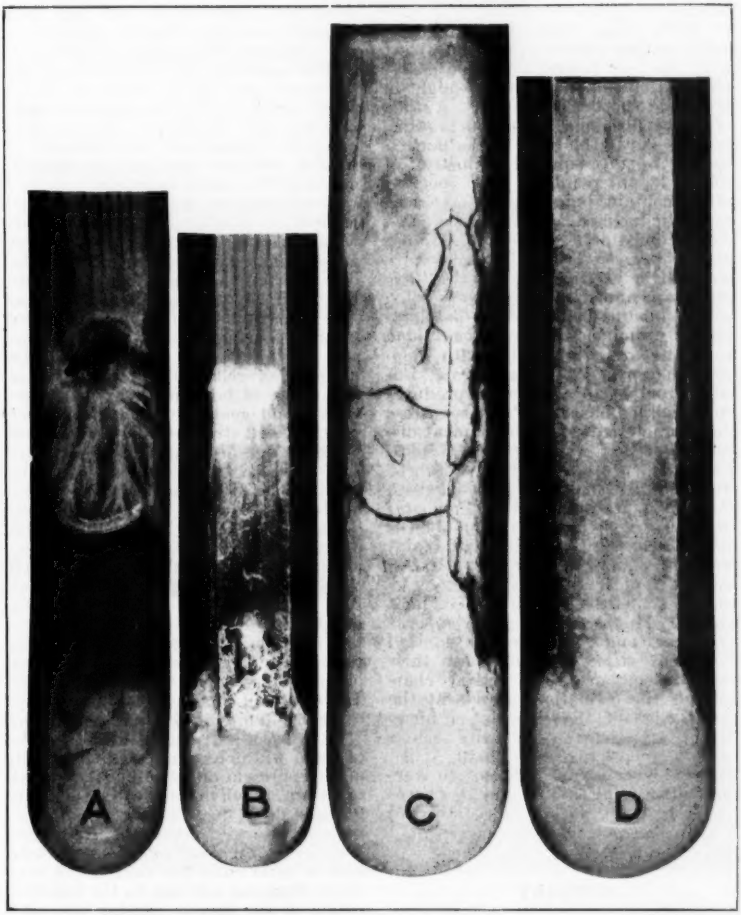
A piece of heartwood oak showing a dark reddish-brown color was submitted for diagnosis. The rot was in the late incipient stage and the gross characters placed it among the brown cubical rots. Microscopical examination showed numerous signs of decay. Clamp connections observed on the hyphae developing in the wood indicated that the fungus was a Hymenomycete. Cultures were made and in a few days mycelium of a bright orange yellow appeared. This was compared with stock pure cultures, and it seemed reasonable to name the causal organism *Polyporus sulphureus*.

A more positive case was a piece of white fir (*Abies concolor*) plank showing a characteristic rot resembling that caused by *Pholiota adiposa*. Cultures on malt agar developed typical though small sporophores of this fungus. In a third case the cultures showed nearly perfect sporophores of *Schizophyllum commune* obtained on malt agar from fragments of infected wood taken from a birch log, and in a fourth case the cultures obtained produced abortive and typical sporophores of *Lentinus lepideus* when fragments of infected railroad ties were used.

The close resemblance of the typical stage of the rots produced by such fungi as *Trametes pini*, *Polyporus anceps*, *Fomes annosus*, *Stereum sulcatum*, *Stereum sanguinolentum*, *Trametes isabellina*, *Fomes nigrolimitatus*, and *Polyporus circinatus* in conifers makes it difficult to determine the causal organism in the absence of sporophores. Cultures aid greatly in this diagnosis and only in the case of *Trametes pini* and *Polyporus circinatus* is there any very close resemblance of cultural characters. In this case careful comparisons will show sufficient differences to distinguish between the two. The cultures of *Polyporus circinatus* on malt agar are a much darker brown than those of *Trametes pini* on the same medium. Other distinguishing characters such as rate of growth, type of surface growth, and discoloration of the medium are to be noted.

Incidentally, the study brings out rather clearly the value of rot characters and cultural evidence in connection with the identification of dubious sporophores of fungi found with the typical rots. The following case is a good example:

Diagnosis was attempted on a section of southern yellow pine fence post with several sporophores of a white polypore attached. The sapwood and part of the heartwood showed large areas of a brown cubical rot. The cultures obtained from the brown rot areas indicated a species of Lenzites (Pl. 10, D), but the sporophores attached showed a white context. *Trametes serialis* was suggested, since it produces a brown cubical rot. However, upon closer examination the early typical stage of a white pocket rot was observed in the sapwood in proximity to the sporophores and merging into the brown rot. The white rot resembled that produced by *Polyporus anceps*. Cultures from the white rot areas, from the sporophore tissue, and from the spores cast by the near-typical sporophore developed on malt agar in the cultures obtained from the brown rot areas furnished sufficient data to identify the sporophore as that of *Polyporus anceps* (Pl. 10, A and B). Sporophores of *Lenzites trabea* were later collected from another part of the same post. These were associated with a brown cubical rot. Cultures of the sporophore tissue were identical with the Lenzites-like cultures obtained as above from the brown rot, and with cultures from the spores cast by the near-typical sporophores developed on malt agar (Pl. 10, D). Therefore in this instance two fungi were isolated and identified from the same sample.



Large test tubes (5×40 cm.) containing test sticks inoculated with wood-destroying fungi.

A.—Pure culture of *P. sulphureus* isolated from oak and inoculated on a stick of *Pseudotsuga taxifolia* heartwood with malt agar at the base. 9/16 natural size.

B.—Pure culture of *P. anceps* isolated from *Picea canadensis* and inoculated on a stick of *Picea sitchensis* heartwood moistened with distilled water. 9/16 natural size.

C and D.—Sapwood and heartwood sticks of *Populus tremuloides* inoculated with a pure culture of the fungus (*Polyporus adustus?*) isolated from the orange-zone white rot areas in sap gum lumber. Note the more luxuriant growth in the sapwood stick "C" and the formation of characteristic orange zone lines in the mycelium. 8/9 natural size.

An interesting problem in identity is furnished by the group of white polypores known as *Polyporus anceps*, *Polyporus ellisianus*, and a collection labeled *Polyporus stipticus*. All three of these fungi were associated with white pocket rots in coniferous woods, and the rots resemble each other so closely that minor differences might be ascribed to host relationships. A study of the typical stage of the rots in radial section shows the ends of the pockets usually roughly squared off instead of rounded or pointed. The pockets often contain black flecks. A characteristic incipient discoloration (fig. 2, A) accompanies *Polyporus ellisianus* in *Pinus ponderosa* (*49*) but is less pronounced or at times lacking in *Picea canadensis* and *Abies balsamea* infected with *Polyporus anceps*. This discoloration is prominent in *Pinus banksiana* and *Pinus resinosa* infected with *Polyporus anceps*. *Polyporus anceps*, isolated from *Picea canadensis*, produces on inoculated blocks of *Pinus ponderosa* the characteristic reddish incipient discoloration followed by the typical white pocket rot. The cultures secured from *Pinus ponderosa* infected with *Polyporus ellisianus* or with *Polyporus stipticus* when inoculated on blocks of *Picea canadensis* produced the faint reddish incipient discoloration followed by the typical white pocket rot. A comparison of the pure cultures on malt agar of *Polyporus anceps*, *Polyporus ellisianus*, and the collection labeled *Polyporus stipticus* brings out their close resemblance. A typical character is the formation, always at the upper edge of the slant, of a finely poroid growth which usually has a narrow smooth margin (Pl. 10, A, B). This evidence seems sufficient to warrant identifying as *Polyporus anceps* the three white pocket rots studied in *Pinus ponderosa*, *Picea canadensis*, and *Abies balsamea*.

SUMMARY

Methods for a complete diagnosis of the decays commonly found in wood and wood products are presented, with the study of a representative number of wood-destroying fungi of economic importance.

Decay and its stages in wood are defined and decay processes considered in relation to the study. A classification under the two main groups of white rots and brown rots and their subgroups is presented as a workable grouping according to gross characters.

The methods useful in the diagnosis of decay are given under the three main divisions of gross, microscopical, and cultural characters. These when carefully determined are believed to furnish sufficient data for the identification of the causal organism.

A comparison of the incipient discolorations and zone lines of various wood rots indicates the value of these gross characters in diagnostic tests. The extent to which hyphae penetrate the wood beyond the discolored areas was determined for some fungi by means of microscopical examinations and by cultures. Studies of the methods of cell-wall penetration, size and shape of bore holes, hyphal characters, and general pathological effect upon the cell structures have furnished evidence of the value of microscopical characters, as has also a critical study of the manner of penetration of the hyphae of *Trametes pini* in pine wood.

Cultural characters are found to be of great value in diagnostic studies, in many cases furnishing the evidence necessary to complete the identification of the fungus decomposing the wood.

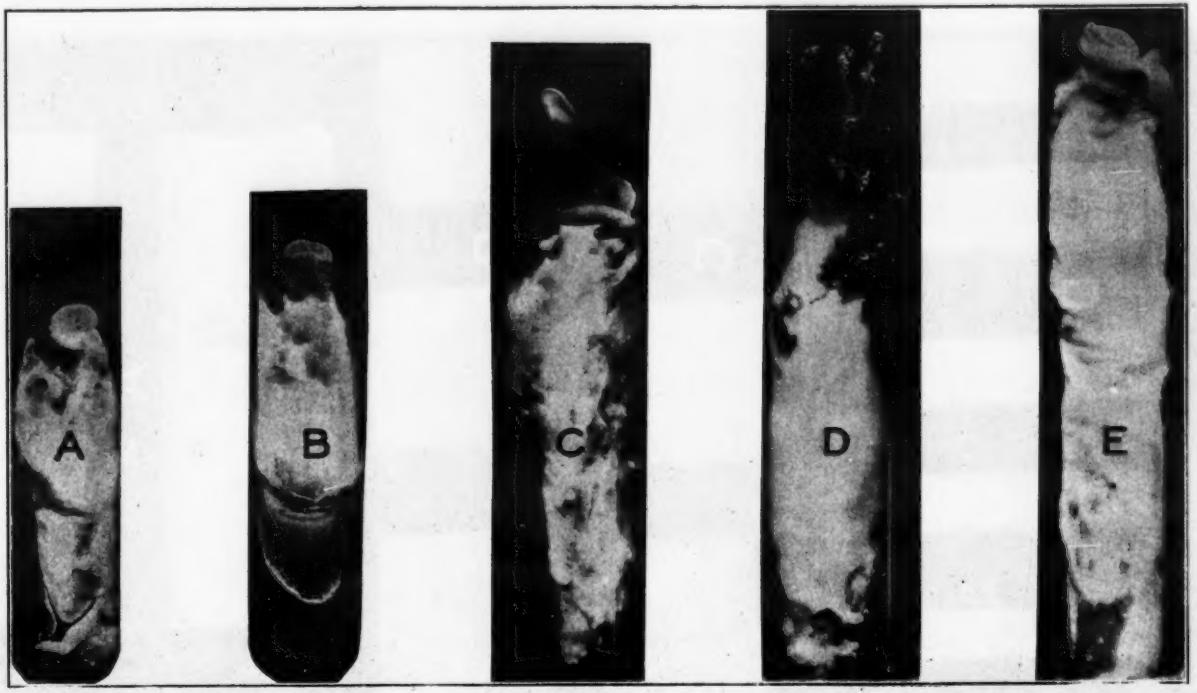
A method is presented which may be used in checking the association of xylophilous fungi with the rots produced. The results of experiments showing this relationship for certain fungi are given.

A complete diagnosis of a sample of wood will determine whether or not decay is present and whether the causal organism is alive or dead, and will furnish data on the nature of the fungus. It will make the detection of decay in otherwise sound wood easier, and in most cases the cumulative evidence obtained will lead to the identification of the fungus causing the rot.

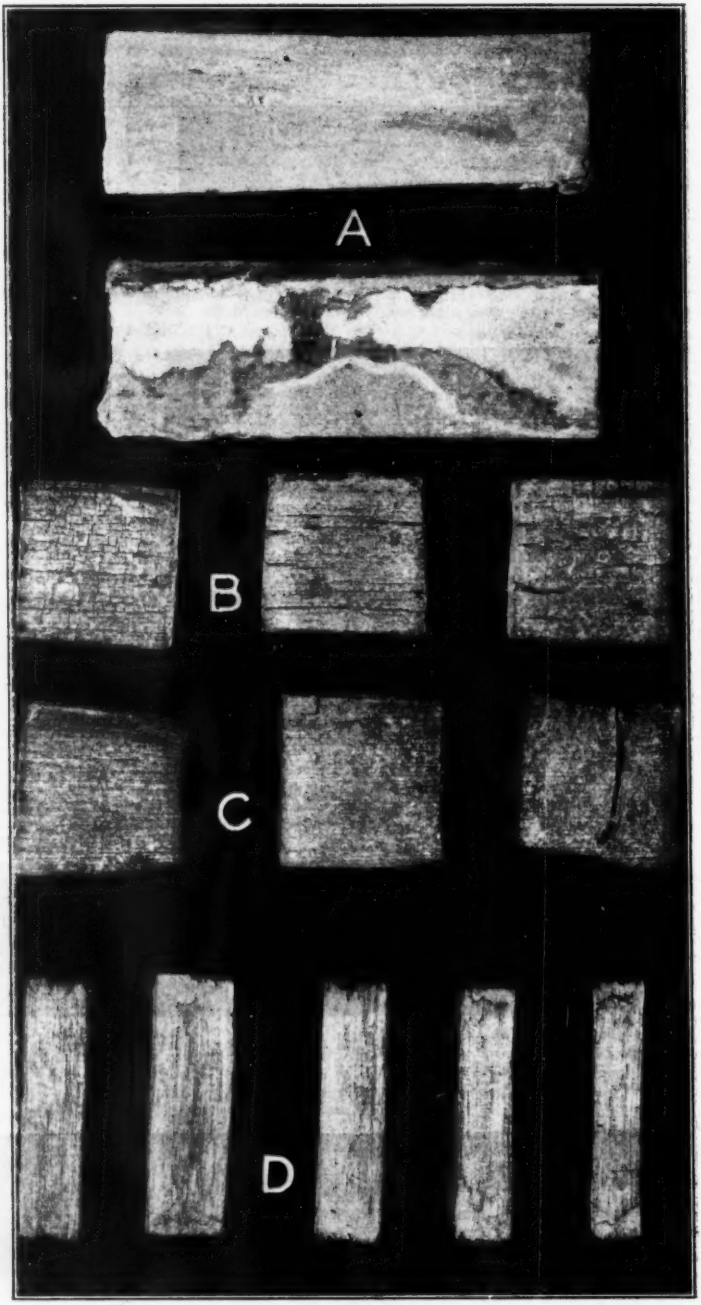
It is believed that use of the diagnostic methods presented will furnish data of scientific value for the solution of numerous problems relating to the staining and rotting of wood and wood products.

EXPLANATORY LEGEND FOR PLATE 10

A and B.—Pure cultures on malt agar of *P. ellisianus* isolated from *Pinus ponderosa* and *P. anceps* from *Picea canadensis*. Note the characteristic poroid growth with smooth margin in both tubes. About natural size. C, D, E.—Types of near-typical sporophores produced on artificial media. ×2. C.—*Lentinus lepideus* isolated from *Pinus banksiana* railroad tie. D.—*Lenzites trabea* developing a hymenial layer which cast abundant mature spores. This fungus was isolated from the brown-rot areas in pine post. E.—*Polystictus hirsutus* isolated from birch wood. Identified by typical sporophores attached to the sample.



(For explanatory legend see p. 562)



The Diagnosis of Decay in Wood PLATE II

(For explanatory legend see p. 565)

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EXPLANATORY LEGEND FOR PLATE 11

A.—Typical rot and zone line produced in *Tilia americana* by inoculation with a pure culture of *Xylaria polymorpha*. B.—Typical rot produced in *Picea sitchensis* blocks by inoculation with *Lenzites sepiaria*. C.—Incipient and typical rot produced in *Picea sitchensis* blocks by inoculation, with *Polyporus schweinitzii*. At center an almost sound block which had been kept too dry at upper part of tube. The block next to this shows heavy infection and great shrinkage at one end which was adjacent to the moist cotton at bottom of tube. D.—Typical white pocket rot and zone lines produced in *Picea sitchensis* blocks by inoculation with *Trametes pini*. Note the zone lines paralleling the ends of the pieces where evaporation was greatest. Natural size.

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TOTAL ASH DETERMINATION IN SPICES[1]

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The chemical determination most widely used by chemists in passing upon the quality of finely ground spices is that of total ash. This is best obtained by igniting one or two grams of the material, contained in a porcelain or platinum crucible, in a muffle furnace at the lowest temperature that will give a carbon-free residue. Heating for one hour at approximately 700° C. is usually sufficient. Red peppers require a little higher temperature than other spices to obtain the same result. Care must be exercised not to use a higher temperature than a dull red heat, or decomposition of carbonates in the ash will result.

Water-soluble and hydrochloric-acid-insoluble ash are determined usually only when the total ash or microscopic examination arouses suspicions of impurity or adulteration. If the total ash is low for the spice under consideration and the water-soluble ash very low the spice has probably been extracted. If the reverse is true of the total and acid-insoluble ash the spice contains extraneous mineral matter.

The color and appearance of the ash often tell something about the spice from which it was derived. Pure red peppers give a light greenish-blue ash, which is due to the presence of copper. American saffron or safflower has a red-brown ash; Spanish saffron yields a white or very pale gray ash; cinnamon ash is white or nearly so, and that of cassia is brown or brownish gray; clove ash is dark green, while most other kinds are white or gray. If spice ash is rubbed between the fingers the presence of foreign mineral matter in the original will be evidenced by a gritty feeling.

In spite of the simplicity of a total ash determination, the figures reported by different investigators vary considerably. A survey of the literature reveals the following partial list of figures as the average percentage of total ash in pure ginger: 4.39, 5.36, 5.55, 6.01, 3.80, 5.27, 3.62, 6.78, 3.66, 5.88, and 4.46. The first four are averages of more than 50 determinations each. Similar variations occur in results reported for other spices. This is chiefly due to variation in the spice itself and to a less extent to the personal equation.

Richardson (*89*) [2] gives the total ash in Acheen black pepper as 8.99 per cent and that of Singapore black pepper as 5.41 per cent. These peppers are obtained from the same botanical species and the difference is largely due to variations in the circumstances surrounding their production, such as soil, climate, and handling.

Almost as large differences occur in spices from the same source from year to year as in those from different sources. This is due to differences in climatic conditions and has been well shown by Sindall (*11*). Table I has been prepared from some of his figures, each of which is the average of a large number of determinations upon pure cinnamon, imported from the same sources in successive years.

TABLE I.—*Per cent of ash in cinnamon*

	1908	1909	1910	1911
China cinnamon	4.79	3.77	3.84	3.27
Batavia cinnamon	4.68	3.79	3.78	4.32

In 1908 the average ash content of all samples from China was higher and in 1911 lower than that of Batavia samples in any year. The averages of the best figures available for four different varieties of cinnamon come between 4 and 4.15 per cent.

This variation is further shown by the percentages following which are the averages presented by numerous investigators for different varieties of black pepper.

[1] Received for publication May 22, 1924—issued February, 1925.

[2] Reference is made by number (italic) to "Bibliography," pp. 572-574. Citations in the bibliography which are not referred to in the text were used in the preparation of the tables.

TABLE II.—*Per cent of ash in different varieties of black pepper*

Batavia	4.93	10.90					
Acheen	8.99	5.14	5.17	4.56	6.44		
Trang	8.85	4.21	4.66				
Singapore	3.48	3.36	5.93	3.20	4.42	5.41	5.39
Tellicherry	5.28	6.41	4.17	4.38	5.35	4.43	
Penang	4.62	6.44	4.02	5.67	4.59	4.74	
Allepo	3.27	4.59	3.85	4.43			

TABLE III.—*Total ash determinations of various spices*

Air-dried spice	Number of samples	Number of analysts	Representative total ash	Total ash permissible under pure food laws (*110*)
			Per cent	*Per cent*
Allspice	296	15	4.51	6
Anise	32	6	6.85	9
Bay leaves	29	5	4.63	
Caraway	51	7	6.73	8
Cardamom fruit	109	12	6.71	8
Cassia	143	4	4.28	5
Celery seed	101	4	8.35	10
Cinnamon	542	19	4.05	5
Cloves	451	13	6.15	7
Coriander	270	4	5.38	7
Cumin	14	4	7.63	8.5
Fennel	48	7	8.12	9
Ginger	841	20	4.89	7
Mace	219	10	2.26	3
Marjoram	156	5	10.62	16
Mustard	151	21	4.83	5
Mustard flour	26	4	5.49	6
Nutmeg	342	8	2.45	5
Onion	14	5	4.28	
Paprika	449	12	6.78	8
Pepper, black	581	23	5.03	7
Do... cayenne	237	15	6.17	7
Do... other red varieties	159	4	6.47	8
Do... white	660	13	1.26	3.5
Safflower	18	4	6.67	
Saffron	156	14	5.38	
Sage	311	2	7.39	10
Savory	29	4	9.94	
Star anise	63	3	2.63	
Thyme	104	4	9.83	14
Turmeric	36	9	6.37	

Similar results have been reported for other spices. It seems useless, therefore, to consider the geographical source of a spice in interpreting a total ash determination except where a consistent difference is well authenticated. Sage seems to be one of the few such spices, for the American-raised variety appears to show consistently a higher percentage of ash than the Austrian.

While numerous figures are available for the percentages of ash in the commoner spices, few reliable results are published for the spices principally used by manufacturers, such as dill, turmeric, fennel, etc.

In order to draw conclusions from a total ash determination, it is desirable to know what percentage should be present when all factors tending to vary it have been eliminated. There are no such figures available at the present time. Each text book and article giving results of ash determinations reports a different figure for the same spice. This is not at all surprising when we consider that most of these figures are averages obtained with a few samples purchased at the same time and in the same market. When we remember further that different degrees of heating will result in different weights of ash from the same sample we understand why some chemists report twice as much ash as others for the same spice.

The percentages for total ash given in Table III are the result of an effort to find the amount of ash in the spices listed when all the conditions affecting

it had been averaged. A list was first made of every available average obtained with goods known to be pure, for each kind of spice. Such figures were obtained from most of the articles listed in the bibliography. A few results which were clearly unreliable were then eliminated, and weighted averages prepared from those remaining. The determinations upon which they are based were made by many different analysts over a period of forty years. Many of them have not previously been published. The unpublished determinations, numbering several thousand, were made chiefly by James Blaine Martin, for the use of whose results grateful acknowledgment is hereby made, and by the author, in the Meat Inspection Laboratory of the United States Department of Agriculture. All the samples were examined physically and microscopically, and no determination on questionable material was used in the preparation of this table.

Variations from these figures should not exceed one-third of their value.

For comparison there is included in this list the maximum total ash permissible by the Bureau of Chemistry in the enforcement of the pure food laws.

The approximate ash content of other spices used to some extent in the powdered form is given in Table IV. It is not claimed that these figures are representative, for they are averages of only a few determinations in each case. However, inasmuch as there are no others available, they are given for whatever value they may have.

The approximate composition of the ash derived from several spices is shown by the composite analyses tabulated below.

TABLE IV.—*Approximate ash content of spices named*

	Per cent
Basil	15.12
Calamus	4.64
Capers	2.11
Cassia buds	4.71
Charlock	6.15
Dill	9.88
Fenugreek	3.01
Garlic	6.43
Juniper berries	2.71
Parsley	11.70
Tarrican	10.43
Vanilla beans	4.78

TABLE V.—*Approximate composition of ash from spices named*

	Black pepper	White pepper	Mustard	Paprika	Cinnamon	Cassia	Cardamon	Marjoram	Fenugreek
K_2O	27.56	6.13	18.90	54.37	14.23	5.55	10.32	19.22	33.20
Na_2O	3.89	.79	.37	3.98	4.02	.91	20.01	.67	5.51
CaO	13.73	32.07	15.57	5.15	39.02	51.30	13.20	20.05	8.57
MgO	7.55	10.58	10.51	6.02	3.35	1.19	4.56	5.67	7.10
Fe_2O_3	.58	2.04	1.09	1.97	.48	6.11	.32	6.68	2.33
Al_2O_3				.09			1.57		
Mn_3O_4	.20	.55			.75	1.18	5.05	.65	.10
CuO				.10					
P_2O_5	9.42	29.54	38.22	16.43	2.97	1.15	6.00	8.98	15.01
SO_3	8.48	3.14	5.76	5.70	2.68	.64	11.74	4.86	7.89
Cl	9.13		.17	3.51	.56	.11	2.35	1.76	4.97
CO_2	12.90	14.81	2.62		31.55	31.02	4.51	7.26	10.66
SiO_2	6.56	.35	6.79	2.68	.39	.84	20.37	24.20	4.66

SUMMARY

1. The ash content of spices is affected by numerous factors, so that analyses made upon samples derived from a particular source and crop are not likely to be representative for that spice in general.

2. Figures representing the percentage of total ash which should normally occur in practically all spices in general use have been presented.

3. Composite analyses of the ash of several spices are given.

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